EFFECT OF SILICON COMPOUNDS ON MICROBIAL TRANSFORMATIONS IN SOIL

by

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In the name of Allah Most Gracious Most Merciful

DECLARATION

I hereby declare, that no part of this thesis has previously been submitted in support of any other degree or qualification at this or any other University or institute of learning.

Fateh Muhammad T. M. Soomro

To my wife,

my daughters,

my brother (Jan Muahmmad),

my niece, my sisters

and my family

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SUMMARY

A study was made of the effects of adding a range of silicon compounds (of potential use as fertilizers) to a variety of different soils. In addition the solubilization of insoluble silicon compounds by bacteria and a species of *Penicillium* isolated from ferns growing in walls (as a likely silicon-rich environment) was determined.

The results of the present study show that:

1) Bacteria solubilize rock potash, releasing free silicon into the medium.
2) Growth of a *Penicillium* Sp. *in vitro* increases the solubilization of sodium silicate, but concentrations of free silicon decrease when the fungus is grown in the presence of silicic acid and rock potash, presumably due to Si-immobilization by the fungus.
3) Water-extractable silicon increased when either silicic acid or rock potash was added to all soils, under both aerobic and anaerobic (waterlogged) conditions.
4) Liming increased the release of soluble silicon from sodium silicate, silicic acid and rock potash, the effect being seen in all soil types.
5) Silicic acid generally decreased bacterial numbers in all soils, at least over the first 14 days of the incubation period.
6) Silicic acid and rock potash had no effect on nitrification, while the addition of sodium silicate stimulated nitrate production, this effect is assumed to be largely due to the resultant marked increase in soil pH.
7) Addition of silicic acid and rock potash led to increased sulphur oxidation.
8) The addition of silicic acid to the agricultural loam soil led to a decrease in arylsulphatase and dehydrogenase activity, as well as respiration and soil biomass.

9) Bacteria isolated for the wall-fern rhizosphere solubilized insoluble silicon compounds *in vitro*. Increases in soluble silicon did not however, occur in media when a species of *Penicillium* was grown under these conditions; probably as the result of marked silicon adsorption by this fungus.

CHAPTER ONE

SILICON IN NATURE

CHAPTER 1- SILICON IN NATURE

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1.1 GENERAL INTRODUCTION

1.1.1 SILICON CHEMISTRY

Silicon, after oxygen is the most largely available, non metal, relatively electropositive element in soils, comprising 27.7 wt %. In the universe silicon is seventh in importance, exceeded only by hydrogen (H), helium (He), carbon (C), nitrogen (N), oxygen (O) and neon (Ne). Silicon is present in the sun and stars and is a chief component of aerolites (a class of meteorites). The terms silica and silicon are derived from Latin and German words, *silex, silicis, and silicium* meaning flint (a very hard greyish-black stone). "Silica" is also used as a short convenient designation for " silicon dioxide" in all its crystalline, amorphous, and hydrated or hydroxylated forms (Table 1.1).

Silicon does not occur in the free state in nature, but combines with oxygen to form tetrahedral (SiO_4), the basic structure in silicon dioxide and in the silicates in which the tetrahedra are joined together to form complex chains, rings, ribbons, sheets or three dimensional frameworks. Silicon exists as oxides and as silicates–a compound of silicon, oxygen and some other element or group. It occurs both as amorphous and crystalline forms, as silicon oxides (e.g. sand (silica), flint, quartz, rock crystal, amethyst, agate, jasper and opal and, mineral silicates as a clay, granite, hornblende, asbestos, feldspar, mica etc). In terms of natural silicates, zircon ($ZrSiO_4$), olivine ($9Mg_2SiO_4$. Fe_2SiO_4), and orthosilicates provide the best examples. Silicates are a large group of compounds of

metal ions and negative ions. Alpha-quartz, the most available form of SiO_2 is a major mineral constituent of many rocks, e.g. granite and sand stone. It also occurs alone as rock crystal and an impure form as rose quartz, smoky quartz (red brown), morion (dark brown) amethyst (violet) and citrine (yellow). Opals are very complex crystalline aggregates of partly hydrated silica. Silicon and carbon, the members of Group 1VA of the periodic table, have many similarities in terms of their structure and bonding. Silicon also resembles carbon in a number of closely related compounds. Carbon is the central element of organic chemistry and basis of life, and silicon dominates the inorganic domain of rocks (Pawlenko, 1986).

In contrast to inorganic silicon compounds, there are no naturally occurring organic silicon compounds, all of which have been created in the chemical laboratory (Pawlenko, 1986). Silicone for example, is a polymeric compound of alternate silicon and oxygen atoms in which hydrocarbon groups are linked directly to silicon.

TABLE (1.1) SOME PROPERTIES OF SILICON.

Property	Constant
Symbol	Si
Atomic number	14
Atomic weight	28.086
Melting point (m.p)	1410^0 C
Boiling point (b.p)	2355^0 C
Critical temperature	4920^0 K
Critical pressure	1450 atm
Specific gravity (sp.gr)	2.33 (25^0 C)
Valence	4
Crystal lattice	Diamond

1.1.2 SILICON IN BIOLOGY AND MICROBIOLOGY

Most microbiologists never come across the element silicon, probably because it is thought to be largely biologically unreactive, and is not transformed by micro-organisms (Wainwright, 1997). Scientific interest in the biological role of silicon dates from the early 1900s when silicon was thought to be involved in the synthesis and structure of the connective tissue (Birchall, 1995).

Silicon is essential for the growth of plants, animals, humans and also micro-organisms. Silicon occurs in the body of primitive organisms in large amounts; generally the higher the organism, the larger the ratio of C : Si in the body. Such ratios range from 1 : 1 in plankton to 5000 : 1 in mammals, plants being intermediate, with ratios of 100 : 1 to 500 : 1. Silicon is present in biological systems as a silanate, an ether (or ester-like) derivative of silicic acid which may play a role in the structure of glycosaminoglycans and their protein complexes.

Three types of silicon compounds are found in living organisms:

1) Insoluble silicon polymers (quartz, crystalline and amorphous polysilicon acids).

These compounds cause lung disease (silicosis) when inhaled in large quantities as dust.

2) Water- soluble inorganic compounds

These compounds can easily pass through the cell membrane (ortho- and oligo-silicic acids and their salts) and are rapidly be eliminated from the body.

3) Esters of ortho- and oligo- silicic acids

These can combine with polysaccharides, phospholipids, cholestrol, choline, etc., which are soluble in organic solvents. Silicic acid esters of cholestrol have been isolated from feathers, and galactose silicates can be isolated from the blades of rye (Pawlenko, 1986).

1.1.2.1 Silicon in plants

Silicon, as a mineral substrate, readily absorbed by plants in the form of soil solution or soil water contains, mainly silicic acid (H_4SiO_4). The roots of plants through their interplay with soil minerals play a major role in the solubilization of Si and, hence, its supply in the soil solution.

Silicon in plants most commonly occur in the form of particles (phytoliths). Miyake and Takahashi (1990) have concluded on the basis of the responses of plants grown in nutrient solutions with and without the addition of Si that lack of Si causes deficiency symptoms in the tomato and cucumber, (*Cucumber sativus)*, and marked adverse effects as well on the growth of the soyabean, (*Glycine max*), and strawberry, (*Fragaria xananassa*). These and other such findings have led to Si being regarded as an essential element for higher plants (Werner, and Roth, 1983; Takahashi, and Miyaki, 1990). Living plants, contain orthosilicic acid, (the only silicon form that is absorbed by the roots from the soil), (Epstein, 1994).

Silicon is usually present in plants as opaline but a form of quartz has also been found in the leaves of lantana, sorghum leaf sheath epidermis, strawberries and black raspberries. Silicon occurs in the *Pteridophyta,* including the spikemosses, horsetail and

ferns, and extensively in the spermatophyta, gymnosperms (including conifers) and angiosperms. These groups include a wide range of silicified herbaceous and arboreal series (Parry *et al.*, 1985).

It was reported that Mn in barley leaf tissues of 300-400 ppm on a dry weight basis was toxic when no Si was present in the nutrient solution, but harmless when the solution contained Si at 0.36 mM. A number of positive effects of the high Si treatment have been found on the growth of the plants: greater leaf thickness than that of the low - Si plants, greater dry weight per unit area of leaf, a small but significant added increment in root fresh and dry weight, and a lower resistance of the leaves to wilting.

Silicon is useful to the young plants as a nutrient and is needed for normal growth. For some unexplained reasons, young barley plants appear to be protected from injury by cold water if colloidal silicic acid is present in the culture solution. Sun flowers can be included in the list of the plants, that appear to require silica, the yield of seed from this plant being increased in the presence of silica. Silicon, when distributed through the plant structure, especially in stem, plays a definite strengthening and stiffening role in the following plants:

1- *Equisetum* (horse tail),

These silicon rich plants, were once used to clean teeth and in the kitchen as a "scouring rush" and were used as abrasives, for polishing wood and household utensils. Silicon in *E. arvense* is deposited as long fibers with in the epidermal membrane and is also exuded as worm like projections until the surface is covered with opaline silica.

2- Bamboo

The hardness and stiffness of bamboo can partly be attributed to its fiber structure. Organic matter containing silica gel known as tabasheer, is also found in the hollow stems. This tabasheer was believed to be of value of treating patients, suffering from asthma and tuberculosis. The inorganic part of tabasheer comprises 99.9% SiO_2.

3- Grasses

Many grasses, reeds, and straws owe their weather resistance (e.g. thatching of roofs) to a heavy impregnation with silica. Rice hulls for example contain large amounts of silica. Both the straw and grain of wheat contain silica. A consequence of the silica content of grain, is that beer is essentially a saturated solution of silica. Grasses such as oats and wheat are also strengthened by deposition of silica in specialized epidermal cells.

4- Spiny plants

Some plants produce, pure silica, in spines or spicules. Two south American plants, *Melinis minutiflora* and *Pappophorum silicosum,* form readily detachable spicules contain from 75-84% SiO_2, while the dried flowers contain 7.5 and 10% SiO_2 respectively. Nettles are also reported to have silica in their barbs.

5- Job's Tears

The seeds of this plant (*Coix lacryma* L.), are hard, brilliant, and neatly spotted, and, as a result, are used for beads. The epidermis is so heavily impregnated with silica that opal can be scratched with it.

6- Palms

Leaves of *Palmyra palm* of India, are used as writing paper. Coconut, coco fiber, bass fiber and manila hemp also contain siliceous concretions.

7- Wood

Siliceous concretions in the form of dense silica particles are found within the cells in tropical woods and can cause the blunting of saws and other tools. It has been reported that silica occur in the wood of the Australian turpentine (*Syncarpia laurifolia*) which has world wide reputation for resistance to the marine borer. It was shown that the resistance to the borer was related the high silica content (0.59 % SiO_2) (Iler, 1979).

1.1.2.2 Silicon in Animals

Silicon is known as an essential trace element for the normal metabolism of higher animals. Connective tissues such as aorta, trachea, tendon, bone and skin and its appendages of several animals, are rich in silicon (Carlisle, 1974). Silicon plays a major role in the formation of connective tissue, bone and cartilage and is also involved in many other important metabolic processes. Silicon is also involved in early stages of bone calcification. An adequate silicon diet is essential for the growth of hair, horn and hoofs in mammals, and bird feathers. Silicon deficiency is incompatible with normal growth and skeletal development in the chick such abnormalities being corrected by silicon supplements (Carlisle, 1972) (Fig: 1.1). Chickens maintained on silicon-free feed have malformed feathers, fragile bones, thin legs and smaller combs in proportion to their size. The deficient chicks also exhibit a significantly lower hexosamine content in their articular

cartilage. For normal growth, chickens require only 0.003% silicon in their food (Carlisle, 1972). Silicon deficiency in the rat also results in depressed growth and deformed skull (Schwarz and Milne, 1972).

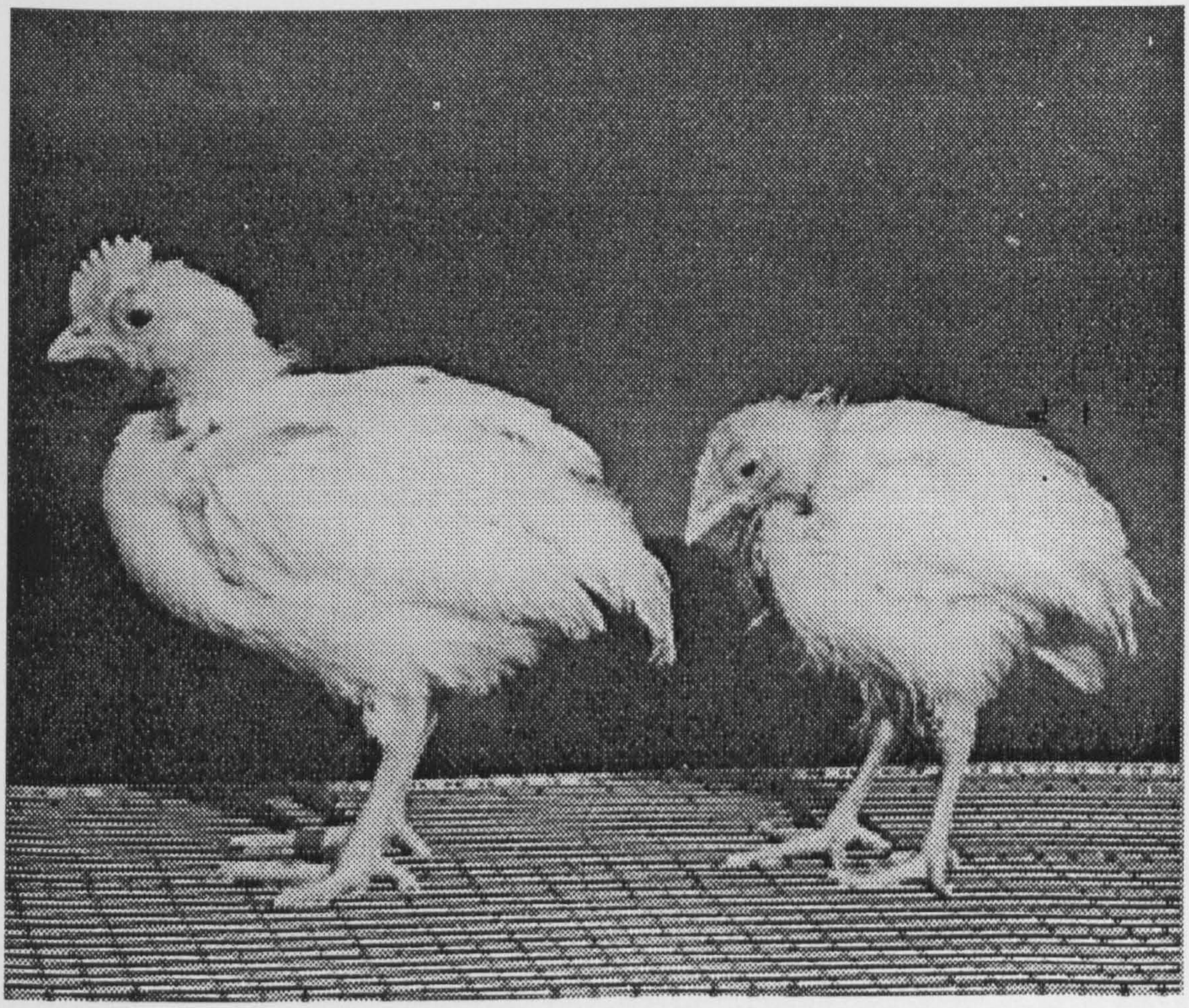

FIGURE (1.1) Four – week – old chicks on silicon – supplemented diet (left) and a low – silicon diet (right) (Carlisle, 1972).

1.1.2.3 Silicon in humans

Nielsen (1988), indicated that the dietary silicon intake of humans varies greatly with the amount, and proportion of the food, of animal (silicon-low) and plant (silicon-high) origin consumed, as well as the amounts of refined and processed foods present in the diet. Normally, refining reduces the silicon content of foods. However, silicate additives are increasingly used (as anti-caking or antifoaming agents) in prepared food and confections. Although an increases in total dietary silicon, is occurring, most of it is not bio available. The human diet, contains around 0.5g of silicon, ingested per day, of which, only 20-30 mg is absorbed through the intestine into the blood stream. The kidneys are responsible for the maintenance of a constant silicon concentration in the blood (Pawlenko, 1986). Normal human serum has a narrow range of silicon concentration, averaging 50 µg/dl (Carlisle, 1986a). The silica content of the pancreas varies with different types of disease. In the case of tuberculosis, the pancreas contains less silicic acid than normal, whereas cases of cancer show more silicic acid than normal. In human optic nerves the silica content increased with age, especially after 60 years, and the silicon content of the skin dermis has also been reported to diminish with age.

The silicon content of drinking water, and beverages shows geographical variation; the concentration of silicon being highest in hard water and lowest in soft water areas. Silicates from foods such as grains or silica supplements (i.e. silicon dioxide or sodium metasilicate) are not directly absorbed by the body. Such silica and silicates must also first be hydrolysed in the stomach to form orthosilicic acid, which is the only form of silicon which is absorbed by the human body (Epstein, 1994).

1.1.2.4 Silicon in micro-organisms

Whether or not micro-organisms contain silica internally or whether it is adsorbed on the exterior is difficult to determine by chemical analysis. Among the many organisms known to be able to utilise silicon (Si) are the silicoflagellida, diatoms and fungi (Honigberg, 1964; Lapo, 1979; Pelczar, 1977; Salle, 1961; Underwood, 1982).

Viruses

Silica is also an essential component of viruses. It has been reported that a crystalline virus consisting of polyhedral particles from lepidopterous larvae (e.g.*Bombyx mori*), contained silicon, (corresponding to 0.2-0.6% SiO_2) as an integral part of the protein matrix (Iler, 1979).

Bacteria

In some soil bacteria, the uptake of silicon as soluble silica in a culture medium is followed by the excretion of phosphorus. Factors which accelerate and inhibit this exchange were studied by Heinen (1963). In the absence of glucose, silicon was lost in the presence of excess phosphate. Particulate fractions isolated from the bacterial membranes were also involved in the metabolism of silicon (Heinen, 1967). The essential role of silica in the metabolism of certain bacteria and the interaction of bacteria and silica gels and minerals have also been extensively investigated, especially in Russia, and have been summarized by Voronkov *et al.* (1975). Bacteria, able to depolymerise crystalline silicates have been reported by Webley *et al.* (1960).

Silicon compounds also increase bacterial growth and have been implicated in aggravating tubercular infection of the lung in patients suffering from silicosis (Price, 1932). The potential importance of microbial silicon transformations in natural environments has been illustrated in a recent paper by Biddle and Azam (1999), who showed that an assemblage of marine bacteria play an important role in the cycling of silica-rich marine diatoms.

Fungi

Certain fungi such as *Aspergillus, Penicillium, Candida, Alternaria, Cladosporium spp.* absorb silica when soluble silicates are added to the culture, a fact which may be due mainly to the adsorption of colloidal silica. However, the fact that in the absence of phosphorus, oxygen accelerates the uptake of silicon suggests that silicon may play a role in the metabolism (Voronkov *et al.*, 1975).

Fungi and bacteria can solubilise silicates, a process which may be important in biological weathering of rocks (Duff and Webley, 1963).

Diatoms

The diatoms constitute the class Diatomaceae or Bacillariophyceae. These absorb soluble silica from water, even at low concentrations, and both metabolise and deposit it as an external skeleton. Nearly all varieties are alike, in that their walls are impregnated with silica. A certain minimum concentration of silica in solution is essential to the growth of each kind of diatom. Increasing silica content from 3.5 to 8.3 ppm doubles the rate of growth of one type of diatom, the dry weight of the cells being between 4-22% SiO_2. When silicon is depleted, cells become coated with a gelatinous capsule of polyuronide or

glucuronic residues. Silica plays a fundamental role in the metabolism of these algae. In the absence of silica, the entire cell becomes disorganized and is unable to keep on dividing (Iler, 1979).

Sponges (Porifera)

Silica from sponges is the source of some silica minerals. The silica content of sponges varies widely from 1 to 90% (Voronkov *et al.*, 1975).

Despite the fact that certain micro-organisms accumulate or adsorb silicon (e.g. diatoms, bacteria, fungi), relatively little is known about its role in the metabolic processes. It seems that silicon is essentially, biological unreactive, but according to science fiction writers, silicon could act as an alternative to carbon, in the biology on other planets. It has occasionally been suggested that silicon may be an alternative energy source, to carbon for microbial life (Das *et al.*, 1992).

1.1.3 USES OF SILICON COMPOUNDS

Various clay minerals are used as raw materials for brick, pottery, earthenware and porcelain tiles. Silica as sand, is a principal ingredient of glass, a material with excellent mechanical, thermal, optical, and electrical properties (Robert and Samual, 1965-66). Hyperpure elementary silicon, when doped with traces of elements such as boron, gallium and phosphorus or arsenic is one of the best semiconductors and is used in transistors, power rectifiers, diodes and solar cells. Elementary silicon is also used in the preparation of silicones (e.g. Me_2SiCl_2) (i.e. organic-silicon compounds), various alloys of iron, aluminium, copper and manganese (e.g. ferrosilicon alloy) are used for de-oxidising steel for castings, for introducing silicon into cast iron, for the manufacture of high-silicon corrosion-resistant iron (Duriron, Tantiron containing 14 to 15% Si) which is very resistant to corrosion and is used in acid-resistant pipes, tanks, for laboratory drains, and for electrical-grade silicon-steel laminations, for electric motors and transformers (Rochow, 1973). Silicones are useful as lubricants, adhesives, fluids, antifoaming agents, elastomers, electrical products and for computer chips (BDH catalogue, 1997). Silicon carbide is one of the most important abrasives (Robert and Samual, 1965-66). Silicon is also used for silicon-based implants in medicine. Soluble silicon $Si(OH)_4$ in drinking water is reported to have reduced the death rates in different communities; with high concentrations of dissolved solids decreasing the probability of cardiovascular-renal diseases. Silicon is a safe additive in foods and is permitted for use as an anti-caking agent. The gelatinous material (silica gel) containing some organic matter which is found inside the hollow stems of Bamboo, is known as tabasheer, which has been patented for use in making cracking catalysts and has also been used for treating asthma and tuberculosis. Silicon is

agronomically essential element for sustainable rice production (Savant *et al.*, 1997) and reduce its diseases. Silicon is also used as a fertilizer for a variety of different crops such as rice (Datnoff *et al.*, 1997) and barley.

1.1.4 BENEFICIAL, HAZARDOUS AND PROTECTIVE EFFECTS OF SILICON

- Colloidal silica helps heal wounds by resorption of toxins and also promotes the development of connective tissues (Iler, 1979).
- Silicic acid favours the accumulation and better utilization of calcium, phosphorus, potassium, and magnesium in the plant. The beneficial effects of silica include mobilizing nutrients from soil and improving resistance to insect and fungal attack. The addition of soluble silicate to soil or culture solutions has a beneficial effect where there is a deficiency of plant available phosphorus. Soluble silica (or silicate ion) is adsorbed by certain components of the soil, particularly clays (Iler, 1979).
- Soil can be fertilized with silica solubilized as a complex with ammonium humate, which is analogous to the catecholate complex (Iler, 1979). Availability of silica may be relatively high in soils which are high in humus.
- Siliceous dust breathed in large amounts can cause serious lung disease called "silicosis".
- Rice that has been treated with talc which is held to the surface of the rice grains by glucose to help preserve the flavour, is responsible for high incidence of stomach cancer in Japan (Iler, 1979).
- Silica can exert its toxic action at the site of protein synthesis.
- All the silicogenic powders exert their specific cytotoxic effects by injuring biological membranes (Iler, 1979).
- Silica is also involved in the aging process and causes senile dementia as well as the identical type of deterioration occurring in the brains of younger people as "Alzheimer's disease".

• By being present in the epidermis of certain plants, silicon increased resistance to fungal diseases such as rusts.

• Cereals which are well supplied with silica are more resistant to mildew infections.

• Silicic acid has an importance in increasing the resistance of plants to powdery mildew fungi. In water cultures, a deficiency of silica produces growth depression in rice, oats, barley, maize, cucumbers, tobacco, bush beans, and tomatoes (Iler, 1979).

CHAPTER TWO

SOLUBILIZATION OF INSOLUBLE SILICON IN CULTURE AND SOIL

CHAPTER 2- SOLUBILIZATION AND UPTAKE OF SILICON BY BACTERIA AND FUNGI *IN VITRO* AND IN SOILS

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2.1 INTRODUCTION

A silicon cycle mediated by microorganisms, occurs in the environment, involving the transformations of silicon between insoluble and soluble states (Lauwera and Heinen, 1974). Silica in its polymer form is almost insoluble, but according to present research, bacteria as well as plants are also able to solubilize polymer silica. Webley *et al.* (1960) reported that *Pseudomonas* and other soil bacteria can bring about the destruction of naturally occurring polymer-silica. These bacteria and fungi can solubilize insoluble silicates by producing mineral and organic acids (e.g. 2-ketogluconic acid) and chelating agents (Henderson and Duff, 1963). Most bacteria (e.g. *Bacillus* and *Pseudomonas*) and fungi (e.g. *Aspergillus, Mucor* and *Penicillium*), are able to decompose aluminosilicate minerals and release a portion of the potassium contained therein (Alexander, 1977). Most of these silicate solubilizers are common soil inhabitants, although Russian workers have described a specialized bacterium, *Bacillus mucilaginous.* These bacteria are useful in removing the silicon from low-grade mineral raw materials, like bauxite, and to extract valuable metals from silicate and aluminosilicate ores and minerals (Karavaiko *et al.*, 1988).

Plants and microorganisms are also capable of degrading experimentally polymerized and natural silicon (quartz) into the monomeric form (Henderson and Duff, 1963). Since the 1920s, monomeric silicon compounds are thought to increase the growth

of microorganisms, e.g. potassium silicate enhances the growth of *Bacillus tuberculosis* (Borell, 1922). Silicon, as silicic acid (0.1- 0.6 mM), is one of the main constituents of soil solution and can be regarded as a plant nutrient (Epstein, 1994; Birchall, 1995). Silicon is involved in plant growth, mineral nutrition and the resistance of plants to fungal disease and herbivores (Epstein, 1994).

Soluble silica has a remarkable influence on soil fertility. Silicon fertilization has been reported to result in increased soil exchange capacity, the transformation of P-containing minerals, the formation of aluminosilicates and of heavy metal silicates. All these effects are caused by changes in soil mineral composition resulting from silicates addition (silicon fertilizers) and to the formation of new clay minerals (Matichenkove, 1999). The beneficial effects of silica on the growth of a variety of crops have been shown, including rice, wheat, barley cucumber and tomato. Silicon fertilizers are applied to crops, in several countries to increase productivity and sustainable production (Jian Feng, 1999). Gaspar (1999), mentions that the growth of rice and sugarcane in rotation on organic and sandy soils in south Florida responds positively to applications of calcium silicate slag. Addition of silicate may also have a nutritional effect because it displaces phosphate ions adsorbed in the soil, thus making phosphate more available to the plant (Iler, 1979).

Silicon can control several important diseases of rice, including blast (*Magnaportha grisea*), brown spot (*Cochliobolus miyabeanus*), sheath blight (*Thanatephorus cucumeris*), and leaf scald (*Monographella albescens*) as well as preventing grain discoloration (species of *Fusarium*, *Bipolaris*, and others) (Datnoff, 1997). It has long been known that silicon compounds can stimulate microbial growth. Allison (1968), suggested a possible role of silicon in the energy metabolism in microbial

growth. Reynolds (1909), suggested that silicon might replace carbon in some types of microbial metabolism. Das *et al.* (1992), however, notes that the exact role of silicon in bacterial metabolism and growth remain largely unknown, but silicon can possibly provide an alternative or additional energy source for several simpler forms of life, particularly members of the *Mycobacteria* and *Nocardiae*. These bacteria may scavenge silicon from the media and the environment and utilize it, even in the absence of a carbon source. Silicon can promote the growth of pathogenic bacteria (e.g. the growth of *Staphylococcus aureus* is stimulated when 100 μg silicon ml^{-1} is added to the medium and high silicon concentration in mucous membrane can increase the number of *Pseudomonas aeroginosa*) (Yoshino, 1990). Price (1932), also mentioned that silicic acid and sodium silicate, even in small amounts, increase the growth of *Mycobacterium tuberculosis* and that the addition of sodium silicate also stimulated the growth of *Amoeba proteus*.

Bacillus licheniformis (bacterial spp.) is capable of accumulating silicon from culture medium (Mohanty *et al.*, 1990). Similarly, Mast and Pace (1937) found that *Chilomonas paramecium* will not grow in inorganic solution lacking silicon and also that silicon stimulated starch production, growth and respiration in this organism. Silicon is also useful for diatom growth, building the siliceous frustule surrounding the diatom cell wall.

The aim of the work presented in this chapter was:

To investigate the microbial solubilization of insoluble silicon in culture and soils, under various conditions:

(a) Microorganisms growing in soil and culture under aerobic conditions.

(b) Microbial silicon solubilization in waterlogged soils.

(c) The effect of lime on the aerobic microbial solubilization of silicon.

(d) The effect of different temperatures on microbial silicon solubilization in soil.

(e) The effect of silicon compounds on bacterial numbers in various soil samples.

PLATE 2.1

Wall-ferns (*Dryopteris dilatata*) growing in wall mortar of University of Sheffield, Firth Court, Western Bank U.K (Side view).

PLATE 2.2

Wall-ferns (*Dryopteris dilatata*) growing in wall mortar of University of Sheffield, Firth Court, Western Bank U.K. (Front view)

PLATE 2.1

PLATE 2.2

2.2 MATERIALS AND METHODS

2.2.1 The effect of silicon compounds was investigated on the following materials:

a) Wall-Fern rhizosphere: Fern plants (*Dryopteris dilatata*), growing in the walls of Sheffield University, were studied for the solubilization of insoluble silicon by microbial attack. (see Plates 2.1 and 2.2).

b) Soil types : Four types of bulk soils, deciduous woodland soil (under beech *Fagus*), coniferous soil (under *Pinus pine*), fern soil (Bracken, *Pteridium aquilinum*) and agricultural loam soil were collected from different areas of Sheffield.

2.2.2 Properties of the silicon compounds used in this study

Potassium silicate – $K_2Si_2O_5$ to $K_2Si_3O_7$, may also contain water. Translucent to transparent hygroscopic, glass-like pieces with a strong alkaline reaction. Usually insoluble, or very slowly soluble in cold water.

Rock potash– A natural, crushed rock product-rich in potassium and silicates. Insoluble in water.

Silicic acid– H_2SiO_3. White, amorphous powder. Insoluble in water.

Sodium silicate– $Na_2Si_2O_3$, $Na_6Si_2O_7$ and $Na_2Si_3O_7$, containing variable amounts of water. Colourless, whitish-grey crystals. Largely insoluble in water. Strongly alkaline.

2.2.3 Determination of dry weight

All the solutions (i.e. culture and soil) were filtered through pre-dried (at 60^0C overnight) filter papers (Whatman No.1) and the dry weight was determined after leaving the filter papers at 60 ^{0}C overnight. In all cases, triplicates were used.

2.2.4 Determination of pH

Bacteria and fungi which grew in Nutrient broth and Czapek Dox Liquid medium, and soil suspended in sterile deionised water were passed through filter papers (Whatman No.1). The pH of the filtrates were determined, using glass electrode pH meter.

2.2.5 Method used for the detection of free silicon

Soluble silicon in the solution was analysed colorimetrically by adding, to 1 ml of filtrate: ammonium molybdate (2 ml, 10% w/v), ascorbic acid (2 ml, 5% w/v), oxalic acid (1 ml, 10% w/v), and concentrated hydrochloric acid (5 ml, 1:1 diluted with distilled water), and mixed thoroughly. The intensity of the blue colour was then measured at 600 nm using a spectrophotometer. The concentration of soluble silicon (SiO_2) in the filtrate was then determined by reference to a standard curve (0-50 µg SiO_2 m^{-1}), prepared using EIL silica (sodium fluorosilicate standard 1000 ppm).

2.2.6 *In Vitro* ability of bacteria (isolated from wall fern-rhizosphere) to release soluble silicon when growing in Nutrient Broth, containing rock potash

Small pieces of the fern roots were directly placed on the Nutrient Agar (Oxoid) with forceps (sterile) and incubated at 37^{0}C for 3 days. After incubation, some bacterial colonies were selected and sub-cultured on Nutrient Agar and incubated for three days at 37^{0}C. After incubation, 0.5 g of rock potash was added to the Nutrient Broth (Oxoid) (20 ml), in the test tubes and mixed thoroughly. All the tubes were plugged with cotton bungs and autoclaved at 121^{0}C for 15 minutes. After autoclaving, bacterial cultures were inoculated with the help of wire loop (sterile), and incubated for 3 days at 37^{0}C by shaking, using an orbital shaker at 150 rpm. Controls were set-up, by adding 0.5g rock potash to each tube, containing medium (without bacterial inoculation). On the day of analysis, culture was filtered through Whatman No.1 filter paper and the amount of silicon in the filtrate was determined.

The pH of the bacterial culture was determined as mentioned above.

2.2.7 *In Vitro* ability of fungus (*Penicillium sp.*, isolated from wall fern-rhizosphere), to solubilize, silicic acid, sodium silicate, and rock potash in Czapek Dox liquid medium

Small pieces of the fern roots were placed on Czapek Dox Agar with forceps (sterile) and incubated for 14 days at 25^{0}C. After incubation, some fungal colonies were sub-cultured on 0.5g rock potash amended Czapek Dox Agar Petri plates and incubated at 25^{0}C for further 14 days.

A colony of the fungus (*Penicillium sp)*, was selected, which produced a clear zone of solubilized silicon, on rock potash amended Czapek Dox Agar petri plates. To Czapek Dox liquid medium (100 ml), in 250 ml Erlenmeyer flask (in triplicates), was added, 0.5 g, each of silicon compounds (i.e. sodium silicate, silicic acid and rock potash (crushed in a Ball-mill), mixed and autoclaved at 121^{0}C for 15 minutes. Each flask was inoculated with fungal disc (4mm). Controls were set-up by adding silicon compound (without mycelium), to the medium. All of the flasks were incubated for 7, 14, 21 and 28 days at 25^{0}C by shaking (150 rpm). The contents of the flasks were filtered every week using Whatman No.1 dried (at 60^{0}C overnight) filter papers. The filtrate was then analysed for free silicon. The dry weight was determined by weighing the filter papers on a sensitive scale after drying at 60^{0}C overnight. The pH of the solution was also determined, immediately after filtration, using a glass electrode pH meter.

2.2.8 Release of soluble silicon from deciduous, coniferous, fern and agricultural soils, amended with silicon compounds, under aerobic conditions

Four soil types, (deciduous, coniferous, fern and agricultural loam) were collected from different areas of Sheffield (See page 24).

To a fresh soil (in triplicates) (100 g, sieved, < 4 mm), was added, 1g each of silicic acid and rock potash plus 2 ml deionised water (sterile). The soils were in the polythene bags and mixed thoroughly. Controls were set-up, lacking added silicon compounds. All of the bags were closed with a rubber band, leaving a small hole for air, and incubated at 25^{0}C. The soluble silicon content of the soils was analysed every week for 4 weeks.

To 100 ml of deionised water (sterile), 10 g of soil were added to 250 ml Erlenmeyer flask, shaken for 15 minutes at 70 rpm and filtered through Whatman No.1 filter paper. The filtrates were then analysed for free silicon as mentioned above. The pH was determined as for the soil solutions.

2.2.9 Release of free silicon in deciduous, coniferous, fern and agricultural soils, amended with silicon compounds, under waterlogged conditions

Each universal bottle (25 ml) was filled, to around full, half with soil (sieved < 4mm) and the soil was weighed and added with 0.5g of silicic acid and rock potash (each compound soil^{-1}) and mixed thoroughly. Deionised water (sterile) was then added, until it remained ¼ inch above the soil surface and the samples were incubated at 25^{0}C. The bottle tops were left loose. Controls were set-up, lacking added silicon compounds. (All soil samples were set-up in triplicates). Analysis was conducted every week for 4 weeks. The universal bottles, containing soil mixture were filled with 20 ml of deionised water (sterile), shaken for 15 minutes at 70 rpm, and filtered through Whatman No.1 filter paper. Filtrate (1ml) was then diluted with distilled water in a 50 ml volumetric flask, and shaken thoroughly. Filtrate (1ml, diluted), was then analysed for free silicon, produced under waterlogged conditions. Dry weight and pH were determined as mentioned above.

2.2.10 Effect of lime (calcium hydroxide, $Ca(OH)_2$) on the release of soluble silicon in deciduous, coniferous, fern and agricultural soils, amended with sodium silicate, silicic acid and rock potash under aerobic conditions

To fresh triplicate soil samples (100 g, sieved < 4mm), was added, 1g each of sodium silicate, silicic acid and rock potash, 1g calcium hydroxide and 2 ml of deionised water (sterile), mixed thoroughly, and incubated in polythene bags. Controls were included containing 1g of calcium hydroxide plus 2ml of deionised water (sterile), without added silicon. All of the bags were then closed with rubber bands leaving a small hole for air and incubated at 25^0C for 7, 14, 21 and 28 days, respectively. Filtrate was obtained by adding 100 ml deionised water (sterile) in 250 ml Erlenmeyer flask. 10 g of the soil were added, shaken at 70 rpm for 15 minutes and filtered through Whatman No.1 filter paper. The filtrate was then analysed and the amount of soluble silicon determined. Dry weight and pH were also determined.

2.2.11 Effect of different temperatures on the release of soluble silicon in agricultural soil amended with silicon compounds

To triplicate samples of agricultural soil (100 g, sieved < 4mm), was added, 1g each of sodium silicate, silicic acid and rock potash and mixed thoroughly in polythene bags. A control was used, lacking added silicon and the bags were closed with rubber bands leaving a small hole for air. The bags were then incubated at 15^0C, 25^0C, 30^0C and 37^0C for 14 days. After incubation, the solutions were filtered through a Whatman No. 1 filter paper as described above and the filtrate was analysed for soluble silicon.

Dry weight was determined and the pH of the fresh filtrate was measured by glass electrode.

2.2.12 Effect of silicic acid addition on numbers of bacteria, isolated from deciduous, coniferous, fern and agricultural soils

To fresh triplicate soil samples (100g, sieved < 4mm), in plastic bags, was added, 1g, silicic acid and 2 ml deionised water (sterile) and mixed. A control was set-up, lacking added silicic acid. All the soil samples were then incubated at 25^0C for 7, 14, 21, and 28 days under aerobic conditions.

2.2.12.1 Analysis

To sterile ¼ Ringer's solution (100 ml), in screw capped glass bottles (150 ml), was added 1g of soil and shaken at 70 rpm for 15 minutes, using an orbital shaker. The soil mixture (10 ml) was then transferred to other glass bottles (150 ml), containing 90 ml sterile Ringer's solution, and mixture was shaken by hand for 3 minutes. From the above solution, 0.1 ml, was inoculated on Plate Count Agar, Petri dishes (6 replicates) using a sterilised glass spreader and incubated at 25^0C for 48 hours. After incubation, bacterial counts were determined every week for 4 weeks.

Statistics

Paired two samples t-tests were performed to check whether means were significantly different.

2.3 RESULTS AND DISCUSSION

2.3.1 (A) *In Vitro* solubilization and uptake of silicon by bacteria and a *Penicillium* sp. isolated from the wall-fern rhizosphere

Bacteria and fungi were isolated from the rhizosphere of ferns growing in the mortar of walls around the University (Firth Court) buildings. The ferns were well-established and their growth had caused localised deterioration of the surrounding mortar. The bacteria isolated were mainly Gram positive cocci while the fungi were almost exclusively species of *Penicillium.*

Table 2.1 shows that the all of the bacterial isolates were capable of solubilizing rock potash to release soluble SiO_2; in two out of the four cases, the amount of the free ion released was significantly different from the control value. This silica solubilization was associated with an increase in the pH of the medium (Table 2.2).

The effect of a species of *Penicillium* on SiO_2 concentration in media containing silicic acid, sodium silicate and rock potash is shown in Fig 2.1. In the case of rock potash and silicic acid, fungal growth led to an decrease in the concentration of free SiO_2, while increases occurred when sodium silicate was added, particularly after 7 and 14 days incubation. Where decreased SiO_2 concentrations resulted, the free silicon ion was presumably take up by, or adsorbed on to the surface of, the fungal mycelium. It is not clear why such uptake did not take place when sodium silicate was the added silicon source. It is likely however, that this resulted from a pH effect, since medium containing sodium silicate was markedly alkaline, in contrast to the pH conditions prevailing in the media containing the two other silicon sources (Fig. 2.2). While the fungus acidified the

medium when growing with silicic acid and rock potash, growth in the presence of sodium silicate led to a further increase in the pH of the medium, to a value as high as pH 10.2.

The ability of bacteria and fungi to solubilize insoluble phosphates and silicon compounds is well recognized (Henderson and Duff, 1963). Such solubilization is undoubtedly an important factor in the microbial erosion of rocks and plays an important role in making P and Si available to plants (Webley *et al.*, 1963). It is usually assumed that such solubilization results from the production, by bacteria and fungi, of organic acids, such as citric acid and 2-keto gluconic acids (Webley and Scott, 1963). However, such a view is based on *in vitro* studies, such as the ones employed here, where large amounts of carbon are readily available. It is possible that in the natural environment insufficient carbon would be available to support such organic acid production. However, since rock weathering is often associated with the rhizosphere of plants such as the wall-ferns studied here, or associated with the growth of lichens, it is possible that in these, circumstances, sufficient carbon would be made available to support organic acid production and therefore acid-related P and Si solubilization. In addition, it is likely that chelating agents will mobilise both phosphates and silicon compounds during rock solubilization. For example complex chelating agents (e.g. lichenoic acids) have been implicated in rock solubilization under lichen and mosses growing on rocks.

The solubilization of insoluble materials in brick mortar leads, as was observed here, to its breakdown and to an obvious reduction in its usefulness as a masonry-bonding material. As a result the growth of plants in walls is obviously deleterious, and presents a biodegradative problem.

2.3.2 (B) Solubilization of silicic acid and rock potash in soils under aerobic and anaerobic incubation conditions

The solubilization of silicic acid and rock potash under both aerobic and anaerobic incubation conditions was determined using samples of deciduous, coniferous, fern rhizosphere and agricultural soils.

(a) Aerobic incubation

The general trend seen for all soils was an increase in soluble silicon with increasing length of incubation over the 35 days incubation period, peaking at 28 days and then declining (Fig. 2.3, a-d). The most marked increases in soluble silicon occurred in soils amended with silicic acid. The release of SiO_2 was most marked in the fern soil (Fig. 2.3 c), essentially equal in the coniferous and deciduous soils (Fig. 2.3 a, b) and less marked in the agricultural loam (Fig. 2.3 d).

These changes in SiO_2 concentration in the soil were associated with a general, small increase in soil pH over the incubation period (Fig. 2.4, a-d).

(b) Anaerobic incubation

Under anaerobic conditions, the general trend was a marked increase in soluble silicon release following both amendments after 7 days followed by a decrease and then a further increase after 21 days. This pattern was amazingly uniform for all four soils (Figs. 2.5 a-d). Rates of SiO_2 release were of the order of 10-15 times less in soils incubated aerobically than in anaerobically incubated soils (Figs. 2.3 a-d). Under anaerobic conditions, solubilization was equally great in

coniferous (Fig.2.5 b) and fern-rhizosphere soils (Fig. 2.5 c), and relatively less in deciduous (Fig. 2.5 a) and agricultural soils (Fig. 2.5 d).

Rock potash increases the pH (between 6-7) of deciduous, coniferous and fern soils (Figs.2.6 a-c) but in agricultural soil, rock potash and silicic acid show slight increase than the control (Fig. 2.6 d).

2.3.3 The effect of lime on the soluble silicon content of soils

The effect of lime on the concentration of soluble silicon in the four soils is shown in Fig. 2.7. In all soils and for all added silicon compounds the general trend was an increase in SiO_2, following the addition of lime, with increasing length of incubation up to 14 days, followed by a slight decline. In all soils, sodium silicate addition led to the largest increase in free, water extractable silicon, followed by silicic acid and rock potash. The agricultural loam showed the least increase in soluble silicon following the addition of silicic acid and rock potash, while the largest increase in soluble silicon was seen following the addition of sodium silicate (Fig. 2.7 d). The pH of the limed soils was between pH 7- 8, with the addition of the silicon compounds generally leading to slight decreases in soil pH (Figs. 2.8 a-d). Liming of the agricultural soil led to higher pH values of around pH 12 (Fig 2.8 d).

2.3.4 Effect of temperature on the soluble silicon content of silicon amended soils

The highest concentration of soluble silicon in all soils, and following amendment with all silicon compounds occurred at 15^0C. The concentration of water-soluble silicon then decreased with increasing temperature up to 37^0C (Figs. 2.9 a-c).

Rock potash and silicic acid show remarkable increase in the pH of agricultural soil at 30^0C (Figs. 2.10 a, c) but sodium silicate increases pH at 15^0C (Fig. 2.10 b).

2.3.5 Effect of silicic acid addition on numbers of bacteria in the four soils.

The addition of silicic acid (1g 100^{-1}g $soil^{-1}$) reduced the number of heterotrophic bacteria in the deciduous soil (Fig. 2.11 a) and to a lesser extent in the coniferous soils (Fig. 2.11 b), but increased numbers in the fern rhizosphere and agricultural soils. (Figs. 2.11 c, d).

The general conclusion from these results is that the addition of silicic acid, sodium silicate, and rock potash to the four soils leads to increases in soluble silicon over the incubation periods used. In many cases, the graphs of soluble silicon show a remarkable degree of uniformity over the four soil types, despite the fact that they exhibit different microbial and physico-chemical characteristics. The concentration of free silicon in the four soils following the addition of silicic acid and rock potash will relate to either chemical or microbiological solubilization or a combination of both. In contrast, the concentration of free silicon resulting from the addition of sodium silicate (since it is soluble) will depend on chemical solubility.

Overall, the results give the strong impression that the concentration of free silicon in soils which results from the addition of all of the silicon compounds is more dependent upon chemical than microbial factors. Liming for example, by increasing the pH of the four soils is likely to particularly influence chemical solubilization and the rate of fixation, if any, of silicon to soils. The fact that the soil solution silicon concentration reached a peak at 15^0C following the addition of silicon compounds, in particular is indicative that chemical factors are taking precedent over microbial interactions. If microbial process were the main factor influencing silicon solubility one would expect to see the "normal" biological response (i.e. an increase in solubility with increasing temperature to around 25^0C) followed by a decrease with increasing temperature; the process being denatured at temperatures above 37^0C.

Additives such as lime will have an impact on physico-chemical process in a soil which will also affect microbiological processes. Such considerations emphasize two problems in research of this nature which are also often associated with studying soils. Both relate to the complexity of the environment under study. Thus while aim here was primarily to investigate the influence of microbial process on the solubility of silicon in soils, it is impossible to exclude physico-chemical reactions from this equation. Soil microbiologists are sometimes not equipped to fully understand the chemical physical reactions influencing the availability of silicon in soils. Of course, soils chemists come across the same problem in reverse and so will tend to under-emphasize the impact of soil microbial processes. Such limitations emphasize the desirability of collaboration between scientists possessing a variety of different expert knowledge of soils.

TABLE (2.1) ABILITY OF BACTERIA ISOLATED FROM FERN RHIZO-SPHERE TO RELEASE SILICA FROM ROCK POTASH.

Bacteria (Isolates)	SiO_2 mg ($20\ ml^{-1}$)
1-	1.67 ± 0.26 *
2-	2.27 ± 0.9
3-	1.73 ± 0.17
4-	1.98 ± 0.023 *
Control	1.51 ± 0.01

Means of three replicates (±), * Significant difference from control P<0.05.

TABLE (2.2) pH OF MEDIUM IN WHICH BACTERIA GREW IN THE PRESENCE OF ROCK POTASH.

Bacteria (isolates)	pH
1-	8.7
2-	8.7
3-	8.2
4-	8.7
Control	7.6

FIG: 2.1

Effect of a *Penicillium* sp. on SiO_2 concentration in Czapek Dox medium containing silica compounds.

—□— Control, containing silicic acid (without mycelial inoculant)

(a)—■— Czapek Dox medium, silicic acid and fungus.

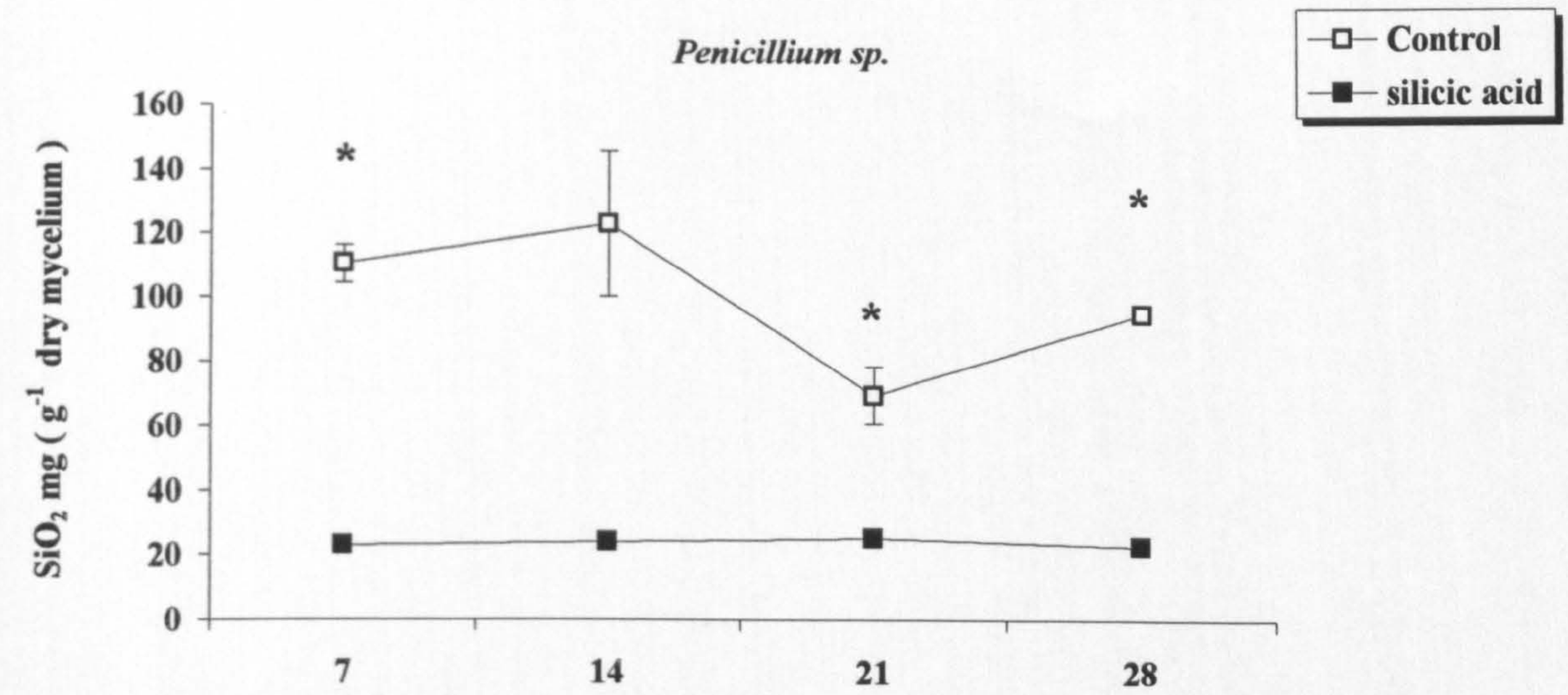
(b)—■— Czapek Dox medium, sodium silicate and fungus.

(c)—■— Czapek Dox medium, rock potash and fungus.

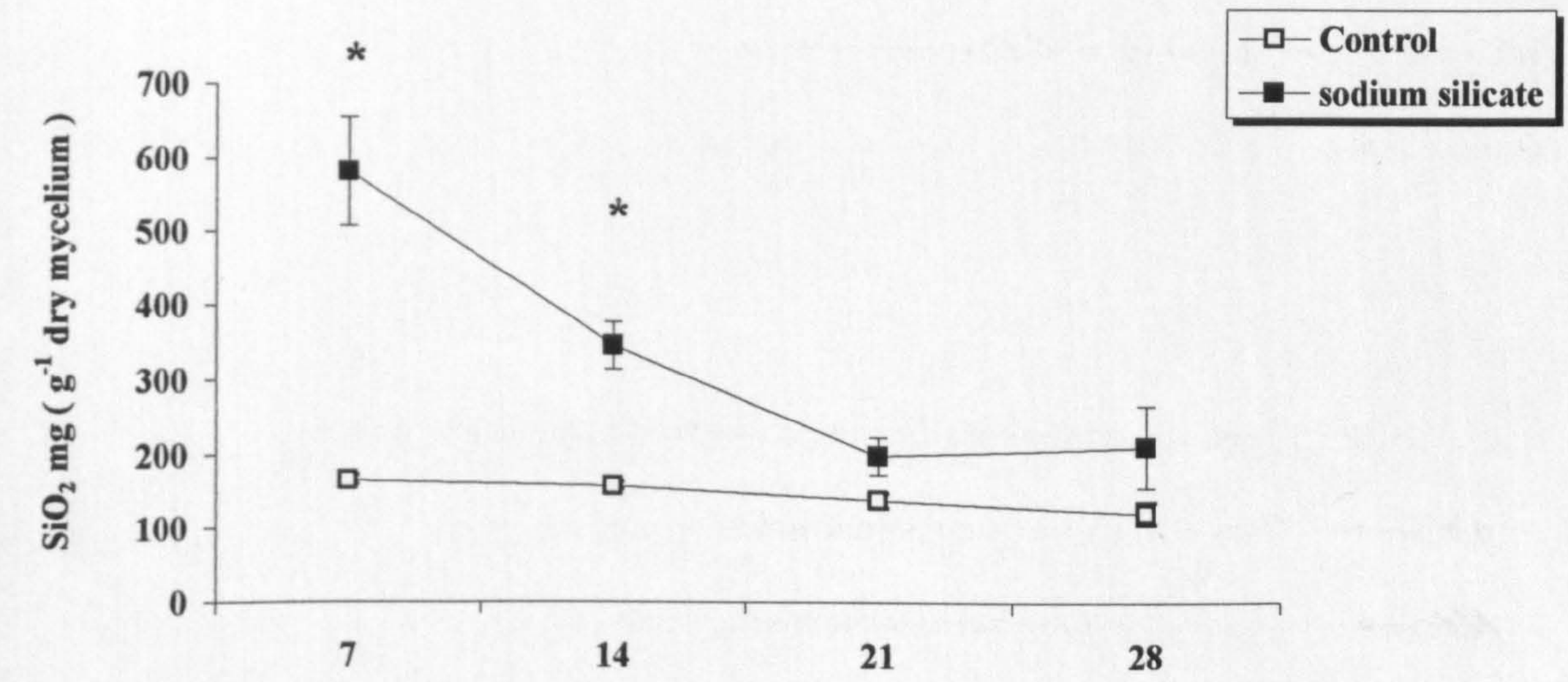
Means of triplicate, ± standard error. *Significant difference from control value, $P < 0.05$.

FIG: 2.1

(a)


Penicillium sp.
Control
silicic acid
SiO_2 mg (g^{-1} dry mycelium)
160
140
120
100
80
60
40
20
0
7
14
21
28
*
*
*

(b)


Control
sodium silicate
SiO_2 mg (g^{-1} dry mycelium)
700
600
500
400
300
200
100
0
7
14
21
28
*
*

(c)

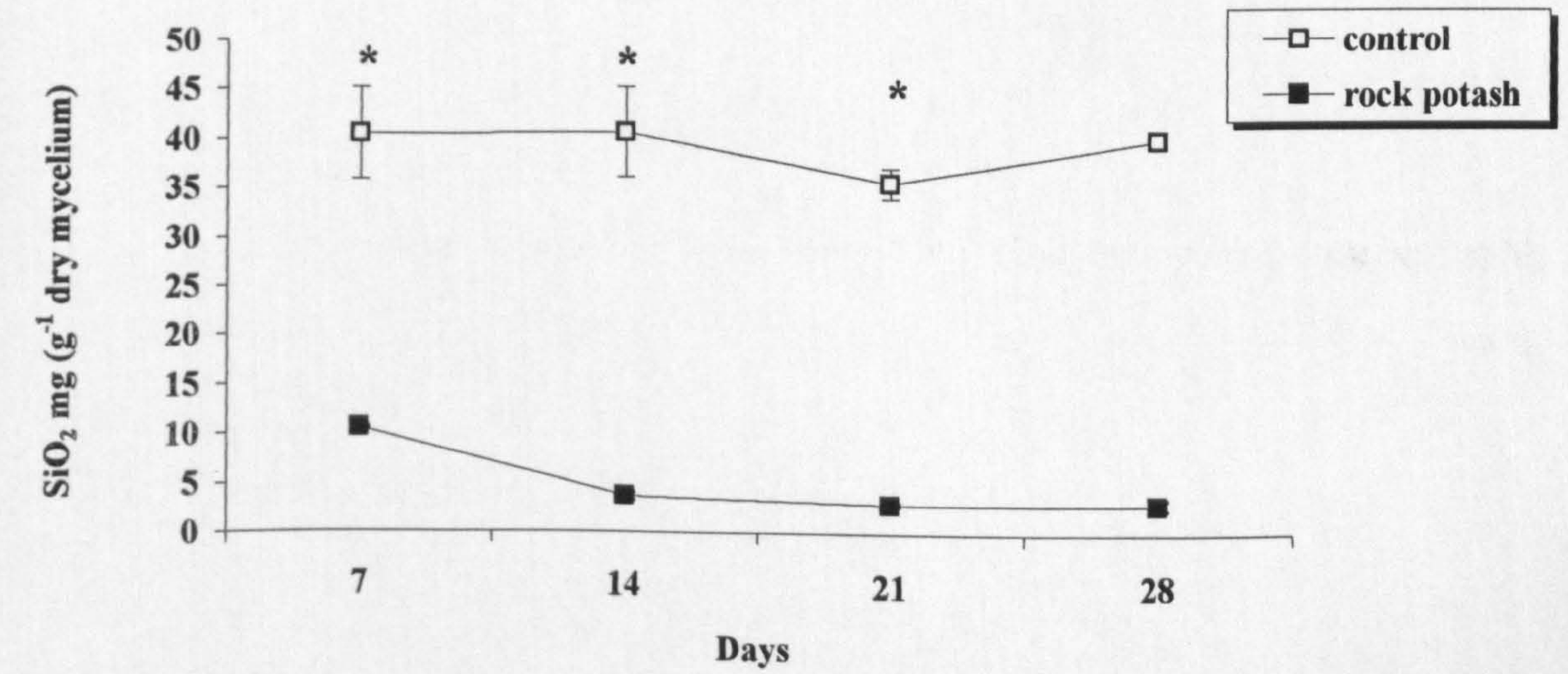

control
rock potash
SiO_2 mg (g^{-1} dry mycelium)
50
45
40
35
30
25
20
15
10
5
0
7
14
21
28
Days
*
*
*

FIG: 2.2

Determination of pH of the medium (*Penicillium*) culture, growing in Czapek Dox medium containing silicon.

—□— Control, containing silicic acid (without mycelial inoculation)

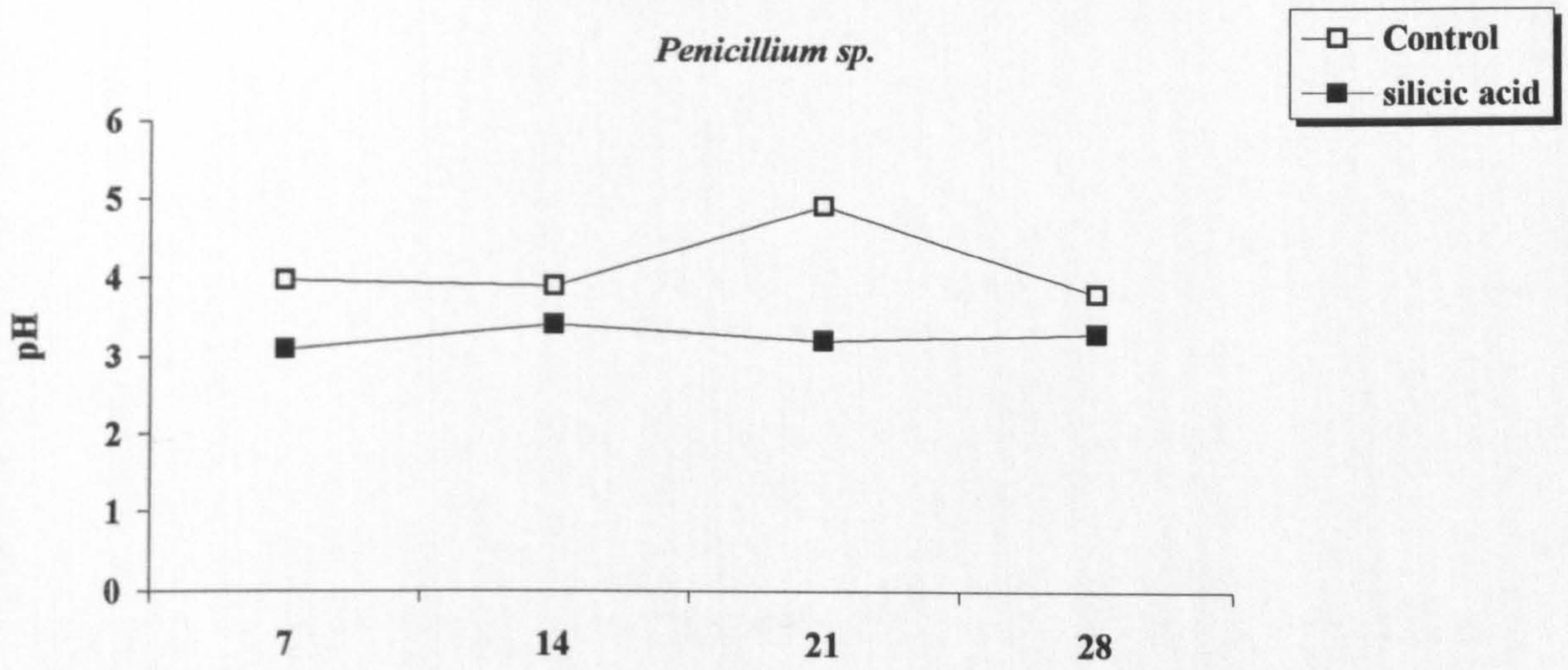
(a) —■— pH of the mycelial culture, added with silicic acid.

(b) —■— pH of the mycelial culture, added with sodium silicate..

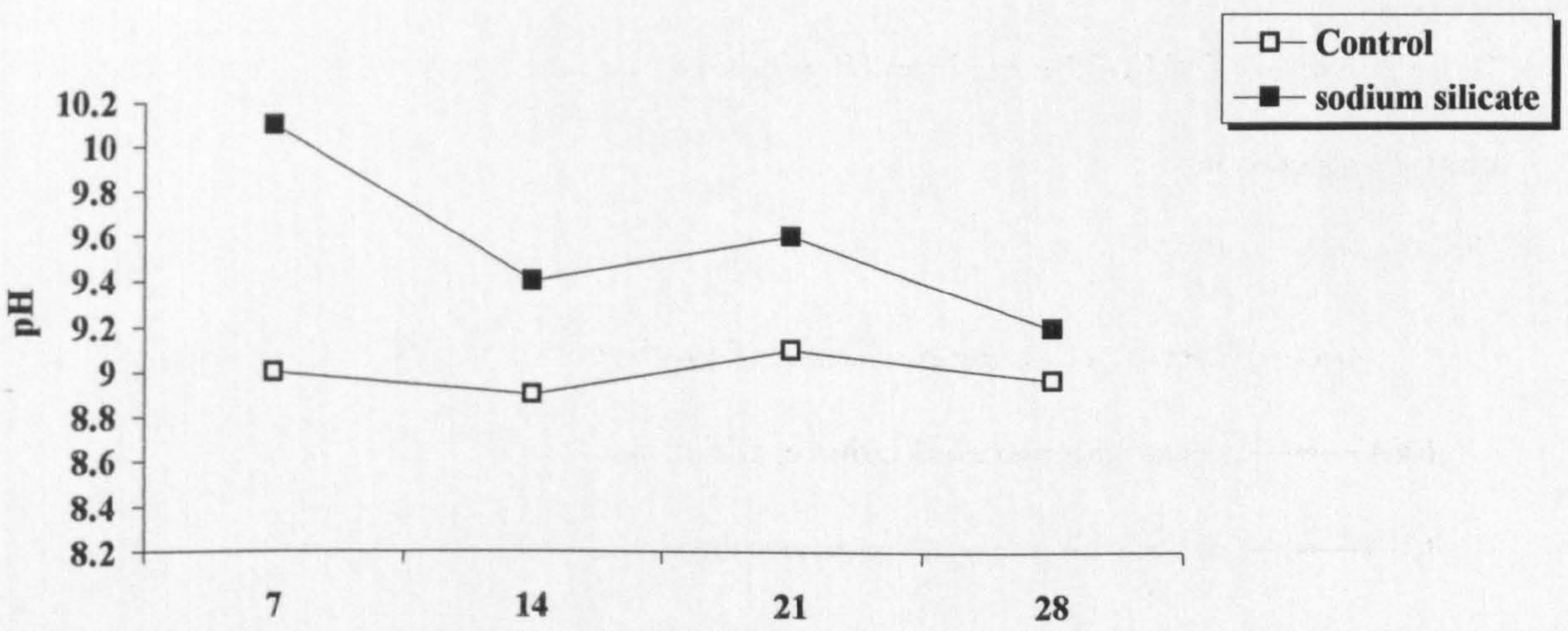
(c) —■— pH of the mycelial culture, added with rock potash.

FIG: 2.2

(a)


Penicillium sp.
Control
silicic acid
pH
6
5
4
3
2
1
0
7
14
21
28

(b)


Control
sodium silicate
pH
10.2
10
9.8
9.6
9.4
9.2
9
8.8
8.6
8.4
8.2
7
14
21
28

(c)

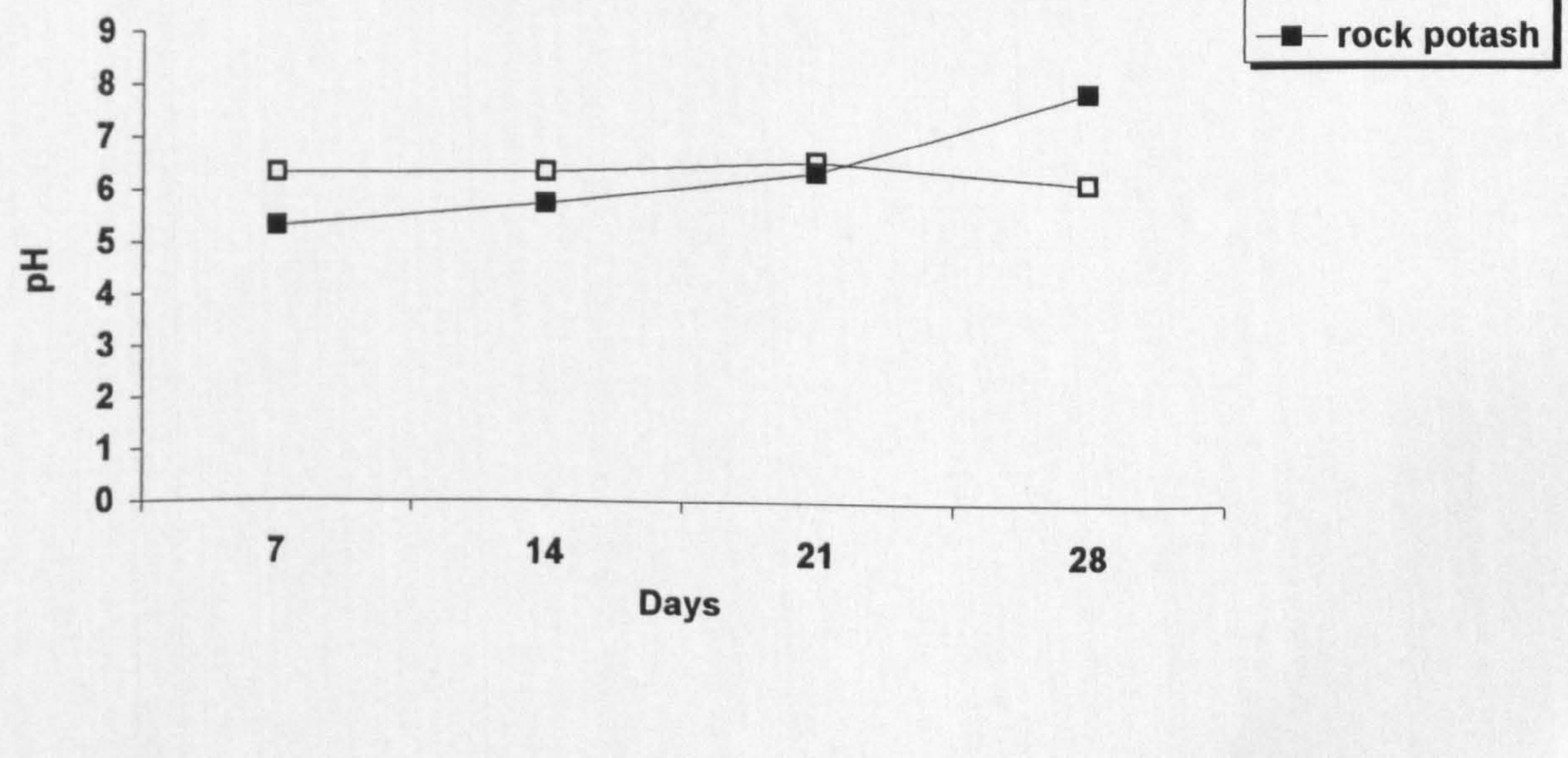

Control
rock potash
pH
9
8
7
6
5
4
3
2
1
0
7
14
21
28
Days

FIG: 2.3

Concentration of soluble silicon in deciduous and coniferous soils, amended with silicic acid and rock potash, under aerobic incubation at 25^0C.

(a) Deciduous soil

(b) Coniferous soil

1- Control (soil lacking added silicon)

2- Soil amended with silicic acid.

3- Soil amended with rock potash

Means of triplicate, ± standard error. *Significant difference from control value, $P < 0.05$.

FIG: 2.3

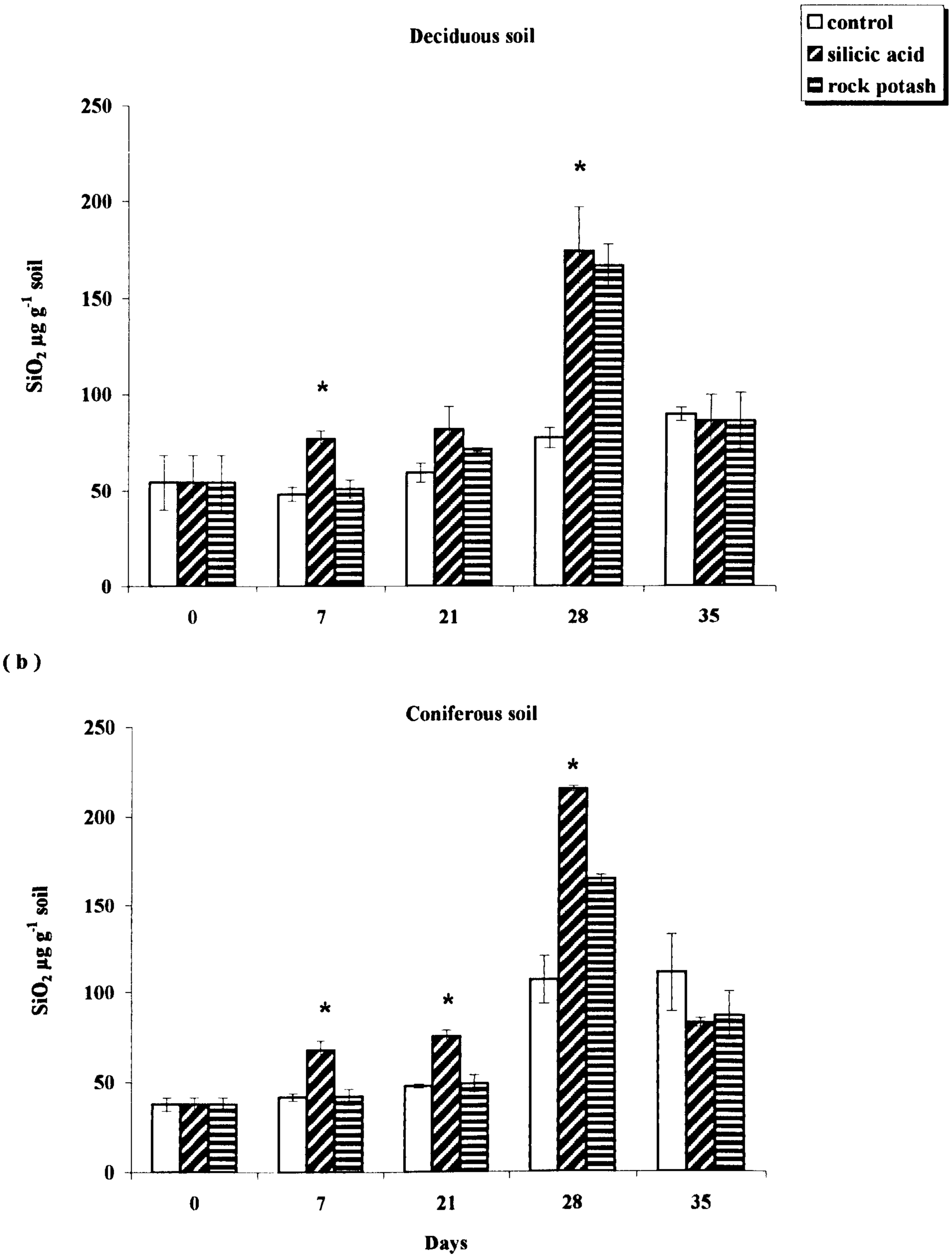

(a)
Deciduous soil
control
silicic acid
rock potash
250
200
150
100
50
0
SiO_2 µg g^{-1} soil
*
*
0
7
21
28
35
(b)
Coniferous soil
250
200
150
100
50
0
SiO_2 µg g^{-1} soil
*
*
*
*
0
7
21
28
35
Days

FIG: 2.3

Cocentration of soluble silicon in fern and agricultural soils, amended with silicic acid and rock potash, incubated at 25^0C, under aerobic conditions.

(c) Fern soil

(d) Agricultural soil

1- Control (soil lacking added silicon)

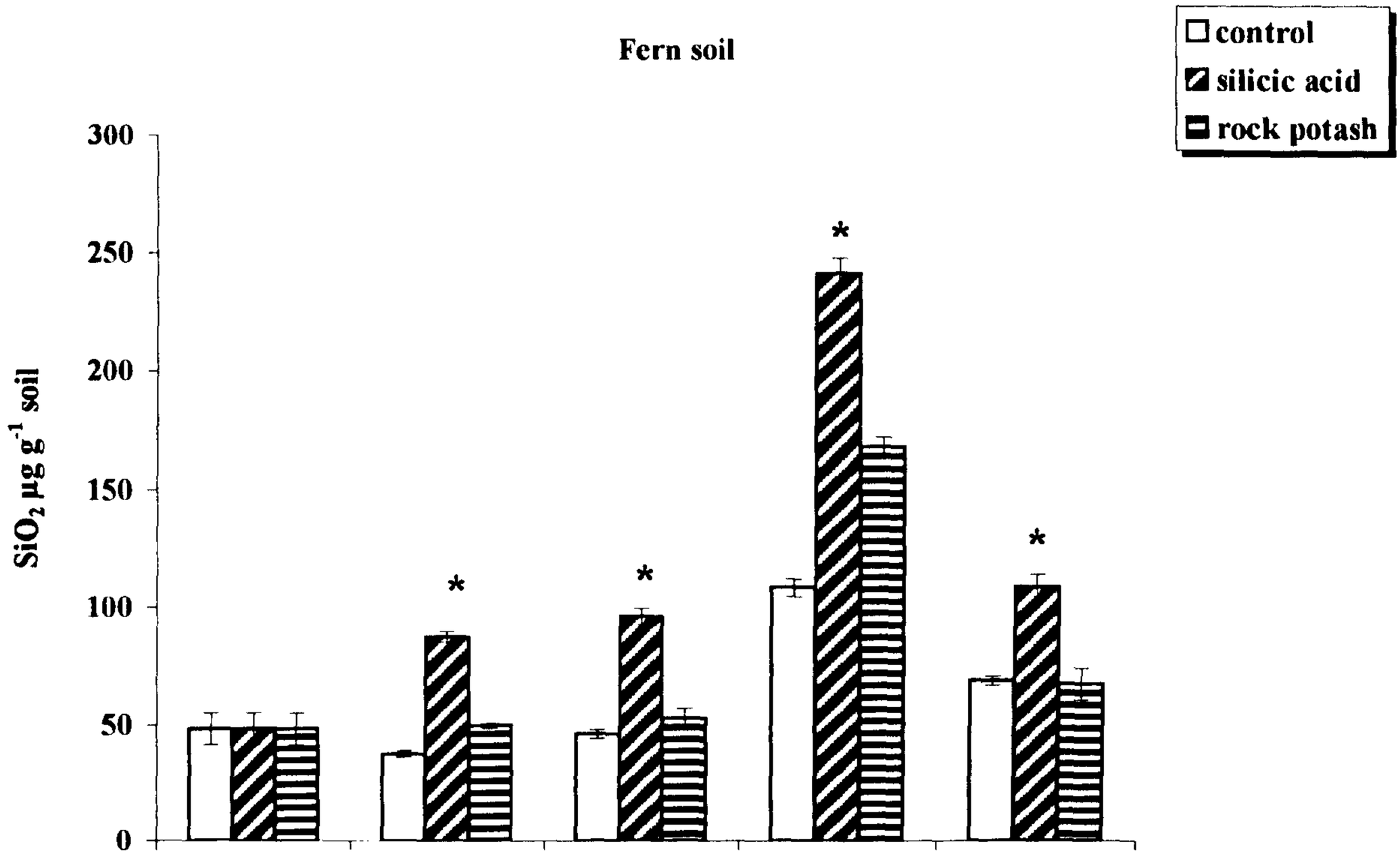
2- Soil amended with silicic acid.

3- Soil amended with rock potash

Means of triplicate, ± standard error. *Significant difference from control value, $P < 0.05$.

FIG: 2.3

(c)


Fern soil
control
silicic acid
rock potash
300
250
200
150
100
50
0
SiO_2 µg g^{-1} soil
*
0
7
21
28
35

(d)

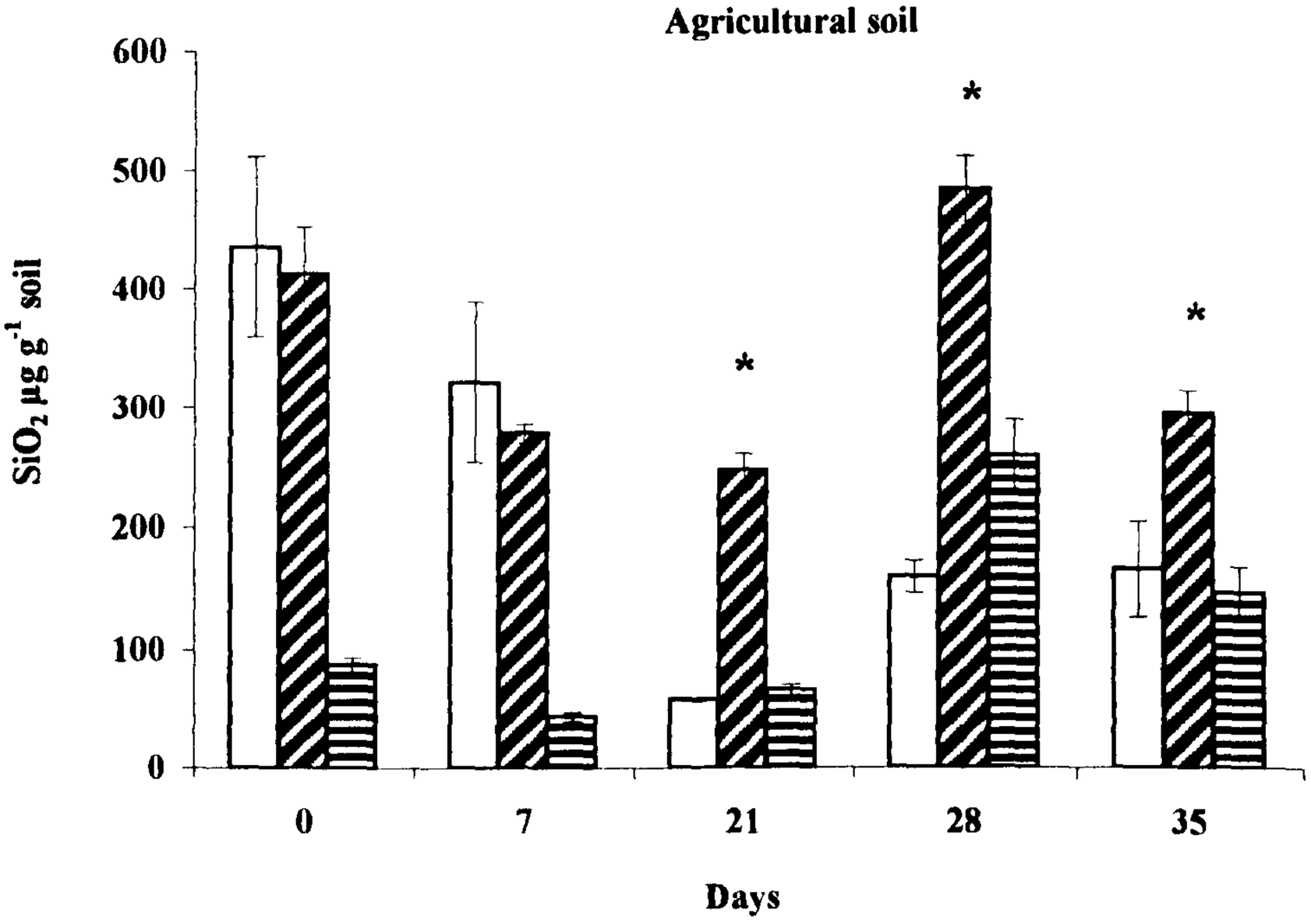

Agricultural soil
600
500
400
300
200
100
0
SiO_2 µg g^{-1} soil
*
0
7
21
28
35
Days

FIG: 2.4

Effect of added silicic acid and rock potash on the pH of deciduous and coniferous soils, under aerobic conditions.

(a) Deciduous soil

(b) Coniferous soil

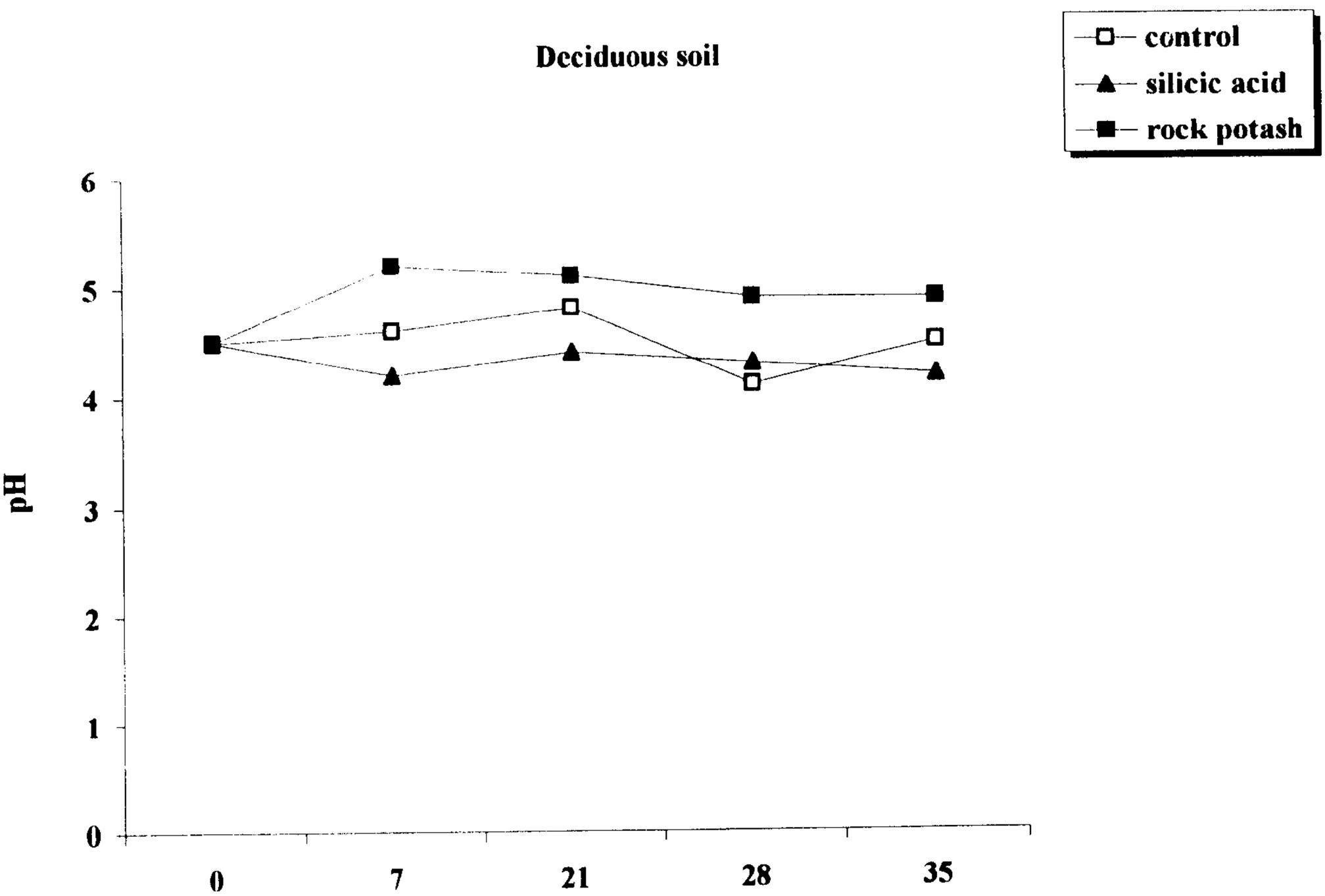
—□— Control (soil lacking added silicon)

—▲— Soil amended with silicic acid.

—■— Soil amended with rock potash

FIG: 2.4

(a)


Deciduous soil
control
silicic acid
rock potash
pH
6
5
4
3
2
1
0
0
7
21
28
35

(b)

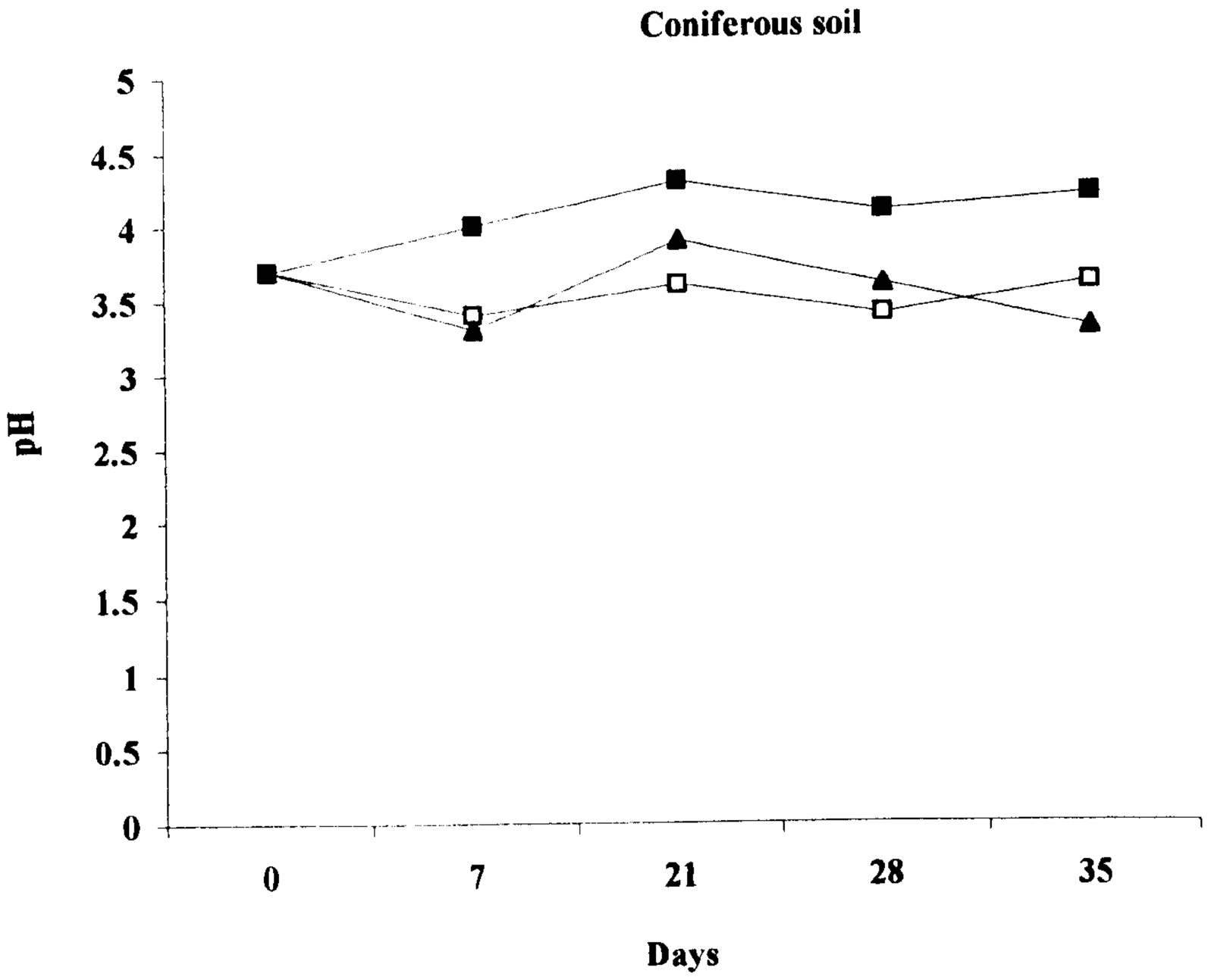

Coniferous soil
pH
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
0
7
21
28
35
Days

FIG: 2.4

Effect of added silicic acid and rock potash on the pH of fern and agricultural soils, under aerobic conditions.

(c) Fern soil

(d) Agricultural soil

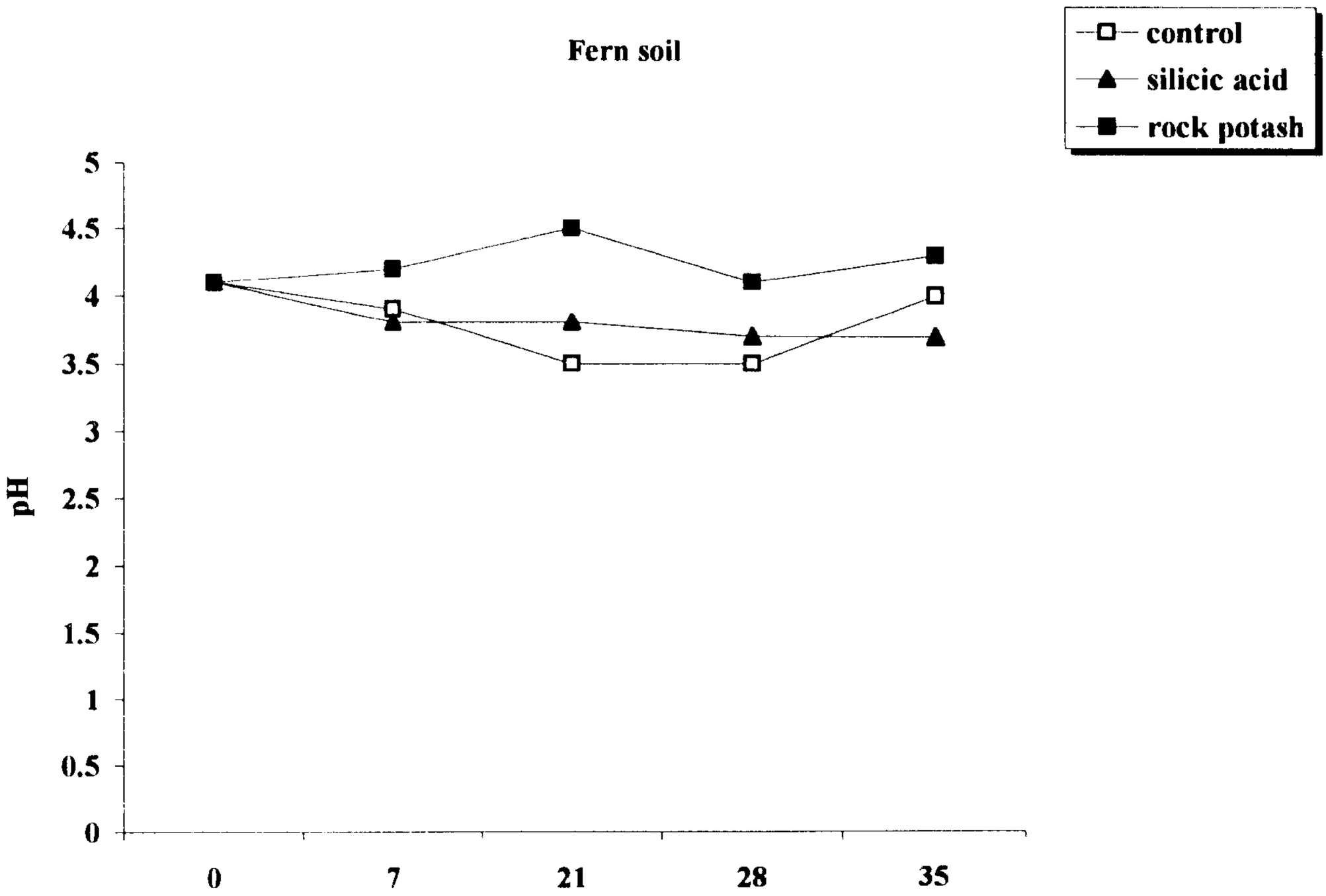
—□— Control (soil lacking added silicon)

—▲— Soil amended with silicic acid.

—■— Soil amended with rock potash

FIG: 2.4

(c)


Fern soil
control
silicic acid
rock potash
pH
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
0
7
21
28
35

(d)

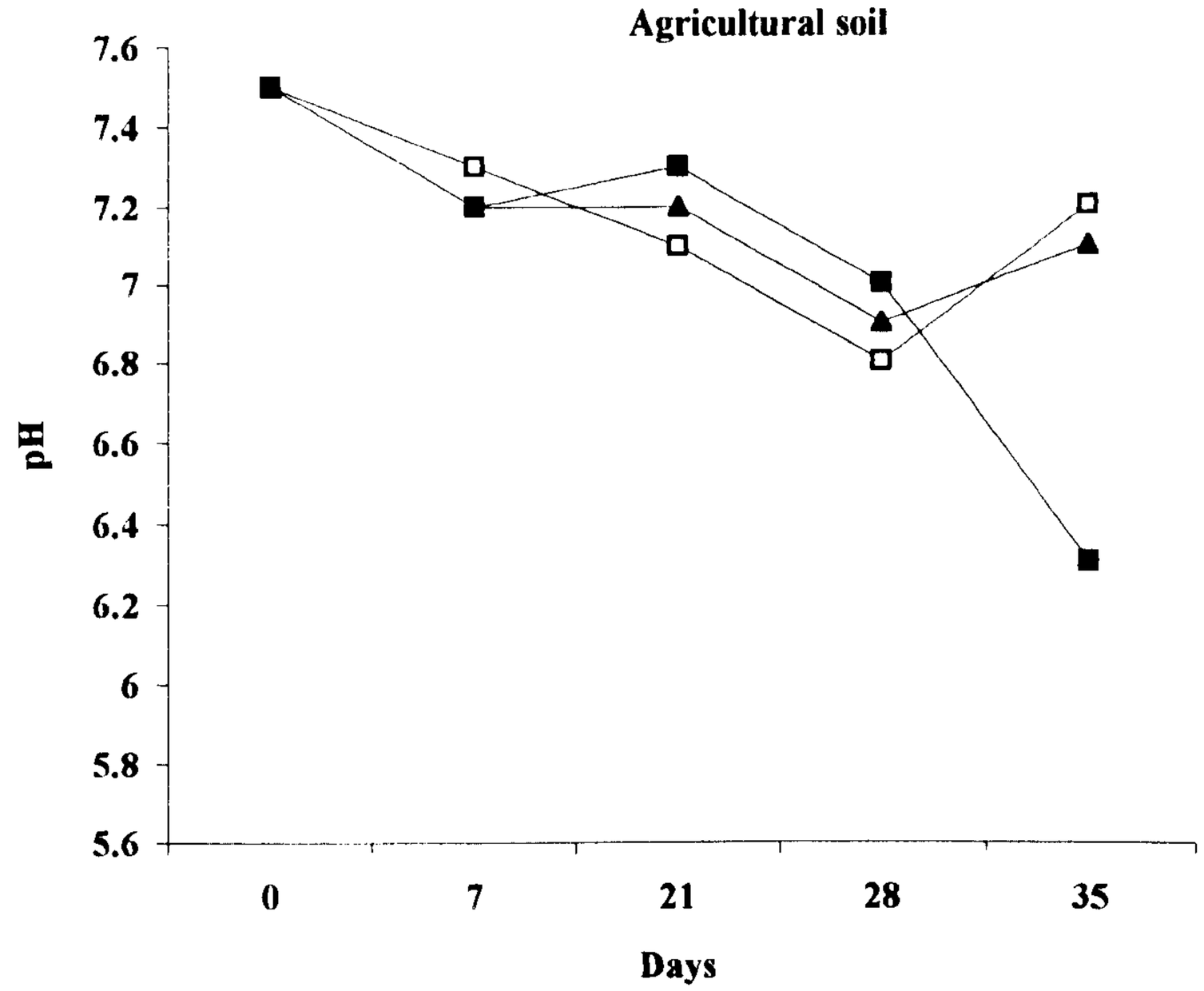

Agricultural soil
pH
7.6
7.4
7.2
7
6.8
6.6
6.4
6.2
6
5.8
5.6
0
7
21
28
35
Days

FIG: 2.5

Concentration of free silicon in deciduous and coniferous soils, amended with silicon compounds, under waterlogged conditions at 25^0C.

(a) Deciduous soil

(b) Coniferous soil

—□— Control (soil lacking added silicon)

—▲— Soil amended with silicic acid.

—■— Soil amended with rock potash

Means of triplicate, ± standard error. *Significant difference from control value, $P < 0.05$.

FIG: 2.5

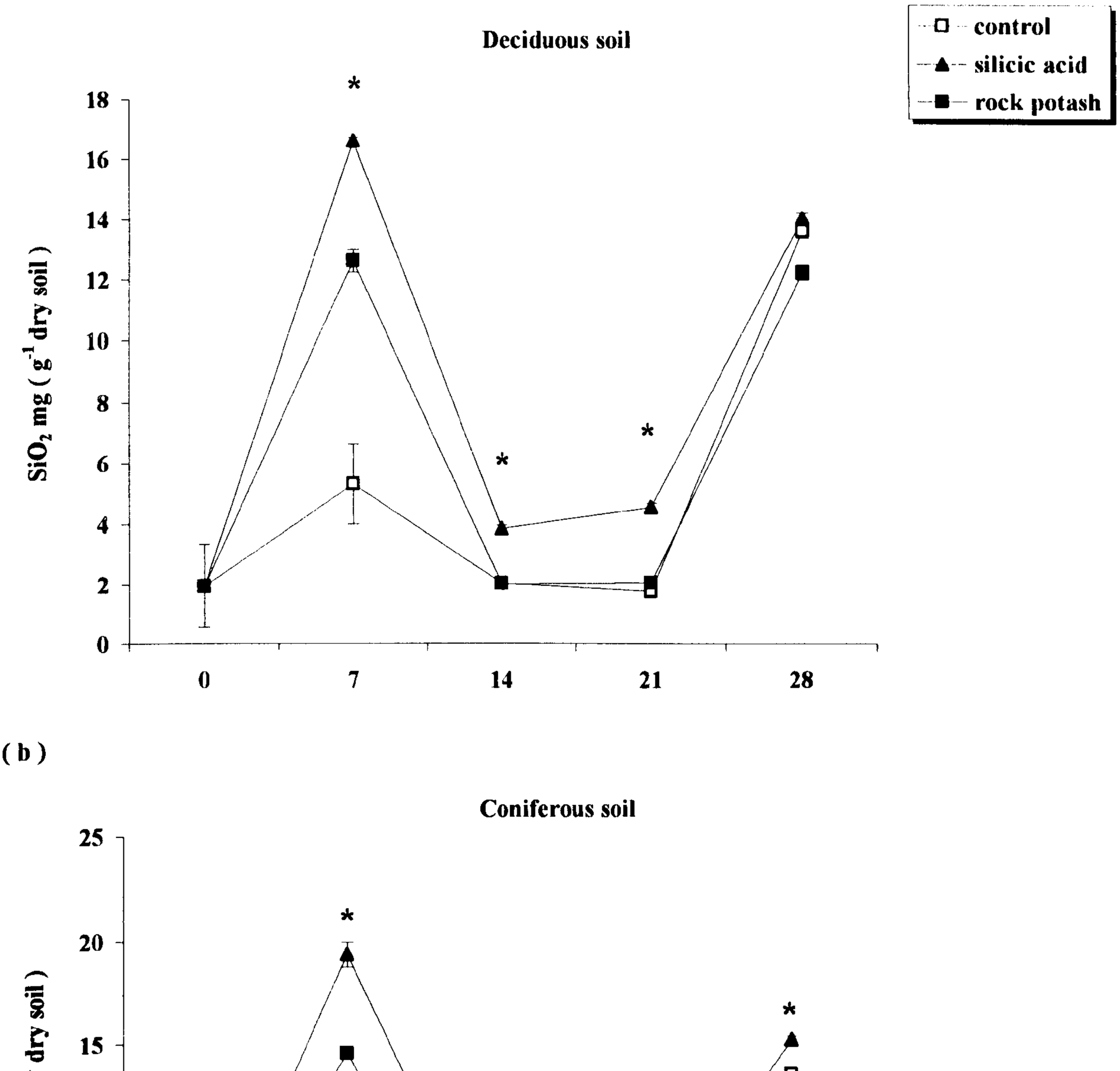

(a)
Deciduous soil
control
silicic acid
rock potash
SiO_2 mg (g^{-1} dry soil)
0
2
4
6
8
10
12
14
16
18
*
*
*
0
7
14
21
28
(b)
Coniferous soil
SiO_2 mg (g^{-1} dry soil)
0
5
10
15
20
25
*
*
*
0
7
14
21
28
Days

FIG: 2.5

Concentration of free silicon in fern and agricultural soils, amended with silicon compounds, under waterlogged conditions and incubated at 25^0C.

(c) Fern soil

(d) Agricultural soil

—□— Control (soil lacking added silicon)

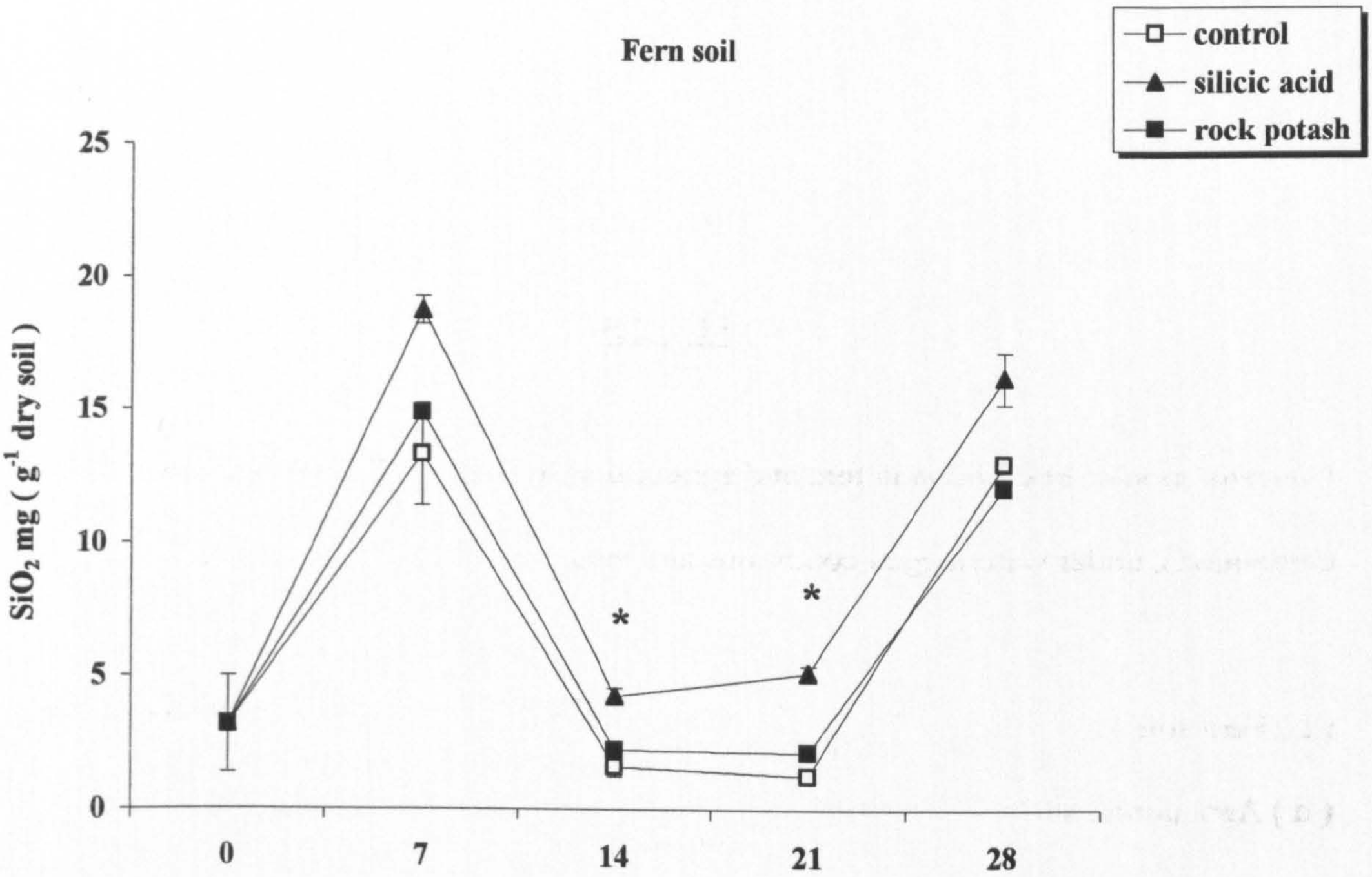
—▲— Soil amended with silicic acid.

—■— Soil amended with rock potash

Means of triplicate, ± standard error. *Significant difference from control value, $P < 0.05$.

FIG: 2.5

(c)


Fern soil
control
silicic acid
rock potash
SiO_2 mg (g^{-1} dry soil)
25
20
15
10
5
0
*
*
0
7
14
21
28

(d)

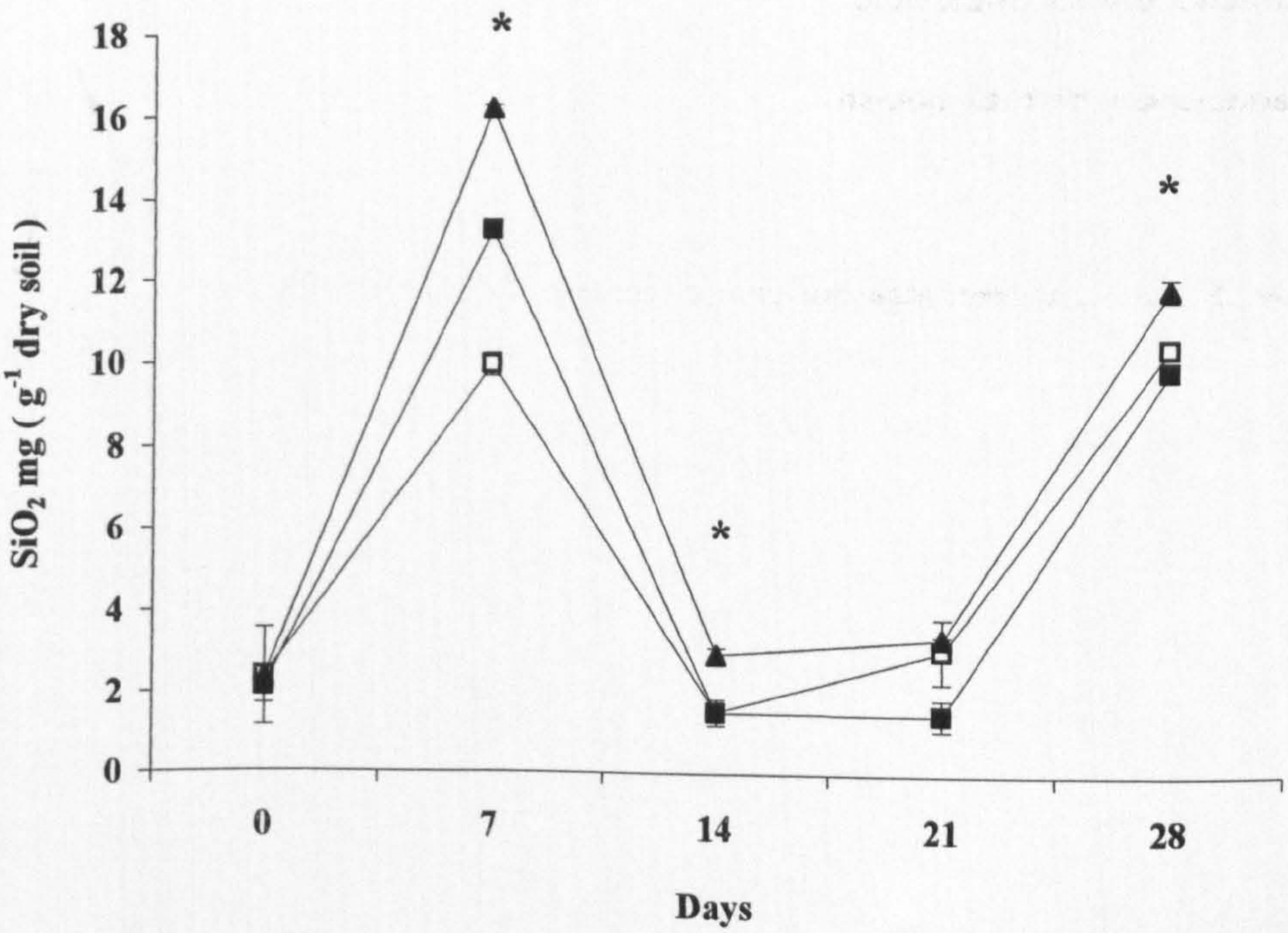

Agricultural soil
SiO_2 mg (g^{-1} dry soil)
18
16
14
12
10
8
6
4
2
0
*
*
*
0
7
14
21
28
Days

FIG: 2.6

Effect of silicic acid and rock potash on the pH of deciduous and coniferous soils, under waterlogged conditions.

(a) Deciduous soil

(b) Coniferous soil

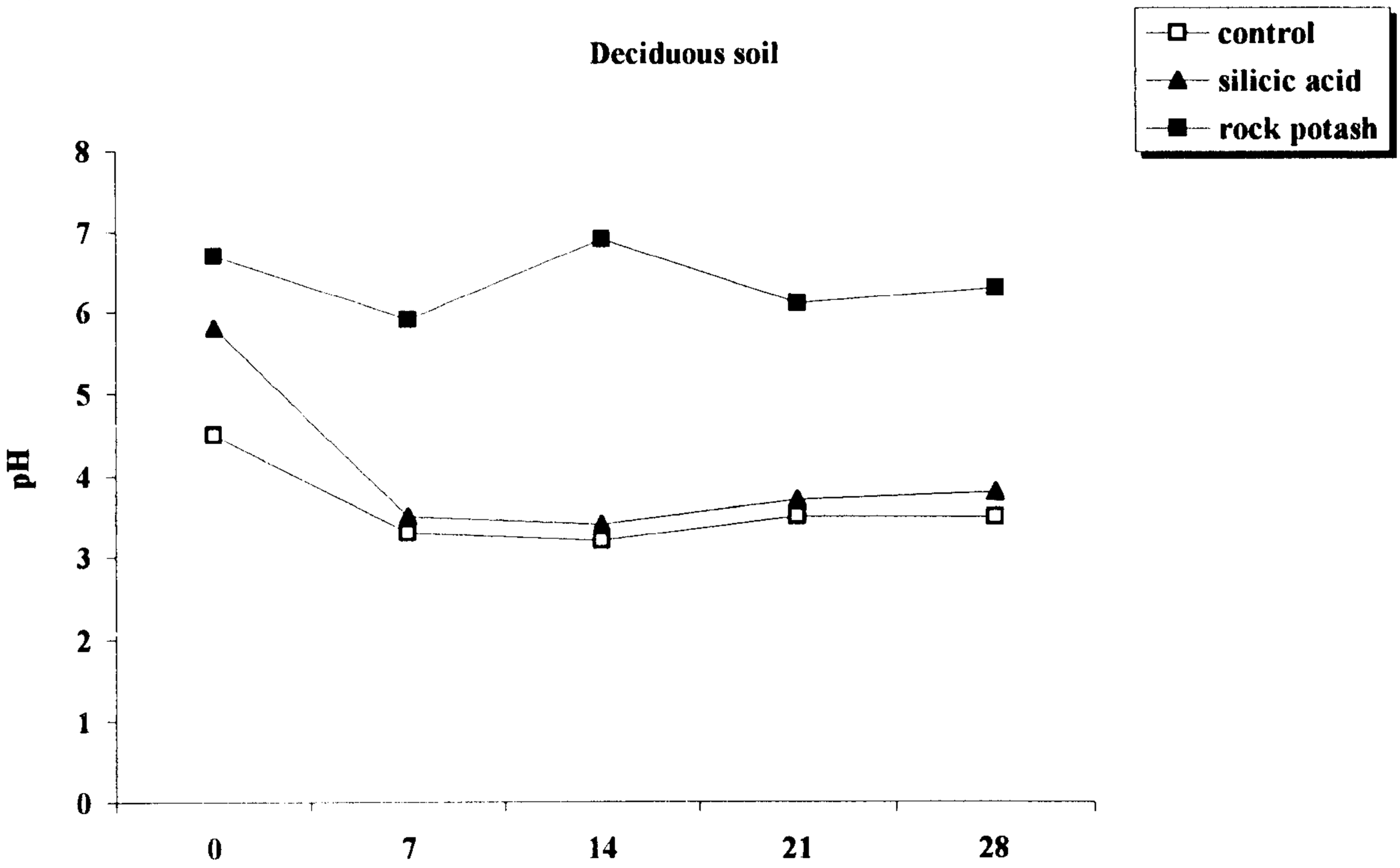
—□— Control (soil lacking added silicon)

—▲— Soil amended with silicic acid.

—■— Soil amended with rock potash

FIG: 2.6

(a)


Deciduous soil
control
silicic acid
rock potash
pH
8
7
6
5
4
3
2
1
0
0
7
14
21
28

(b)

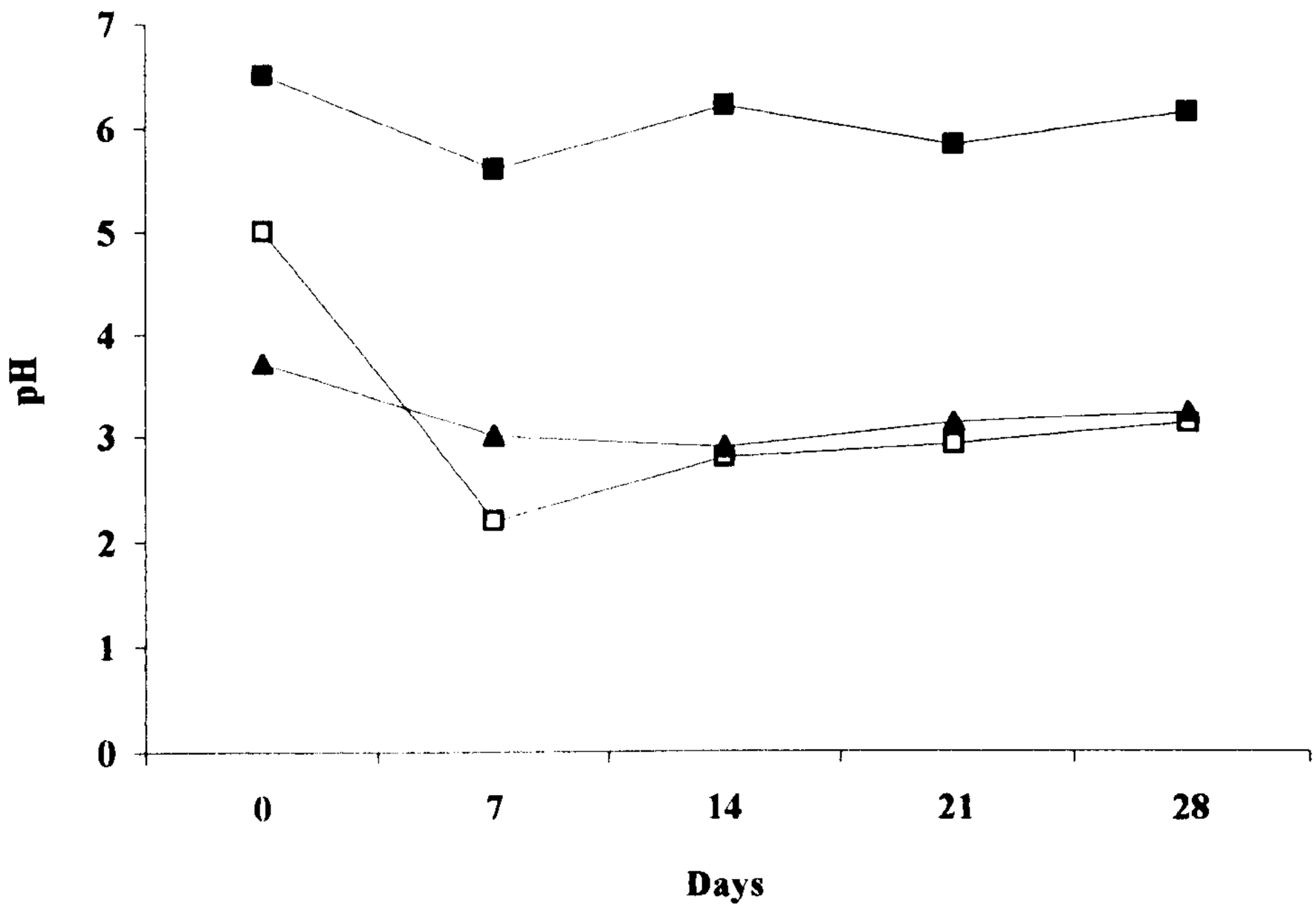

Coniferous soil
pH
7
6
5
4
3
2
1
0
0
7
14
21
28
Days

FIG: 2.6

Effect of silicic acid and rock potash on the pH of fern and agricultural soils, under waterlogged conditions.

(c) Fern soil

(d) Agricultural soil

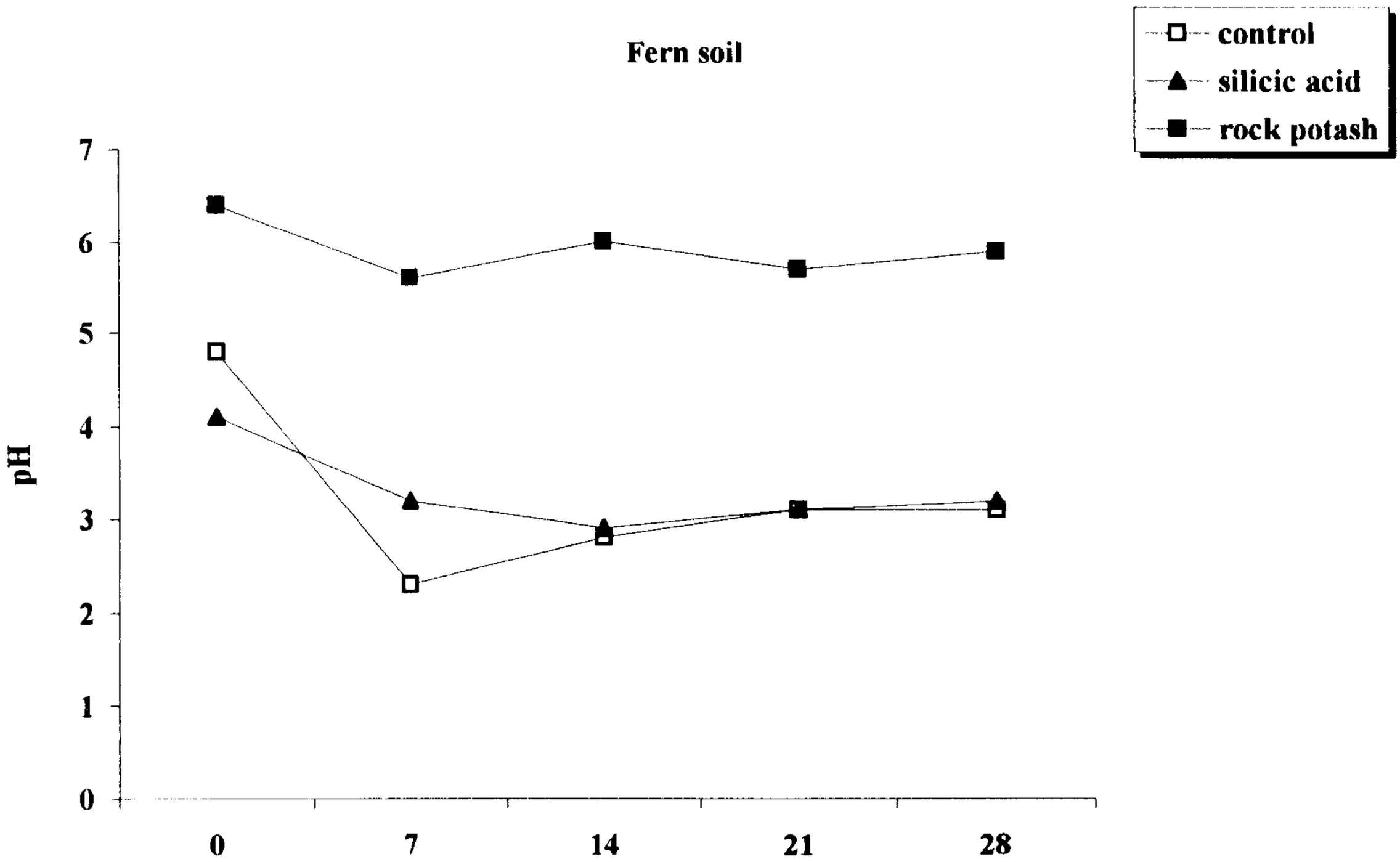
—□— Control (soil lacking added silicon)

—▲— Soil amended with silicic acid.

—■— Soil amended with rock potash

FiG: 2.6

(c)


Fern soil
control
silicic acid
rock potash
pH
7
6
5
4
3
2
1
0
0
7
14
21
28

(d)

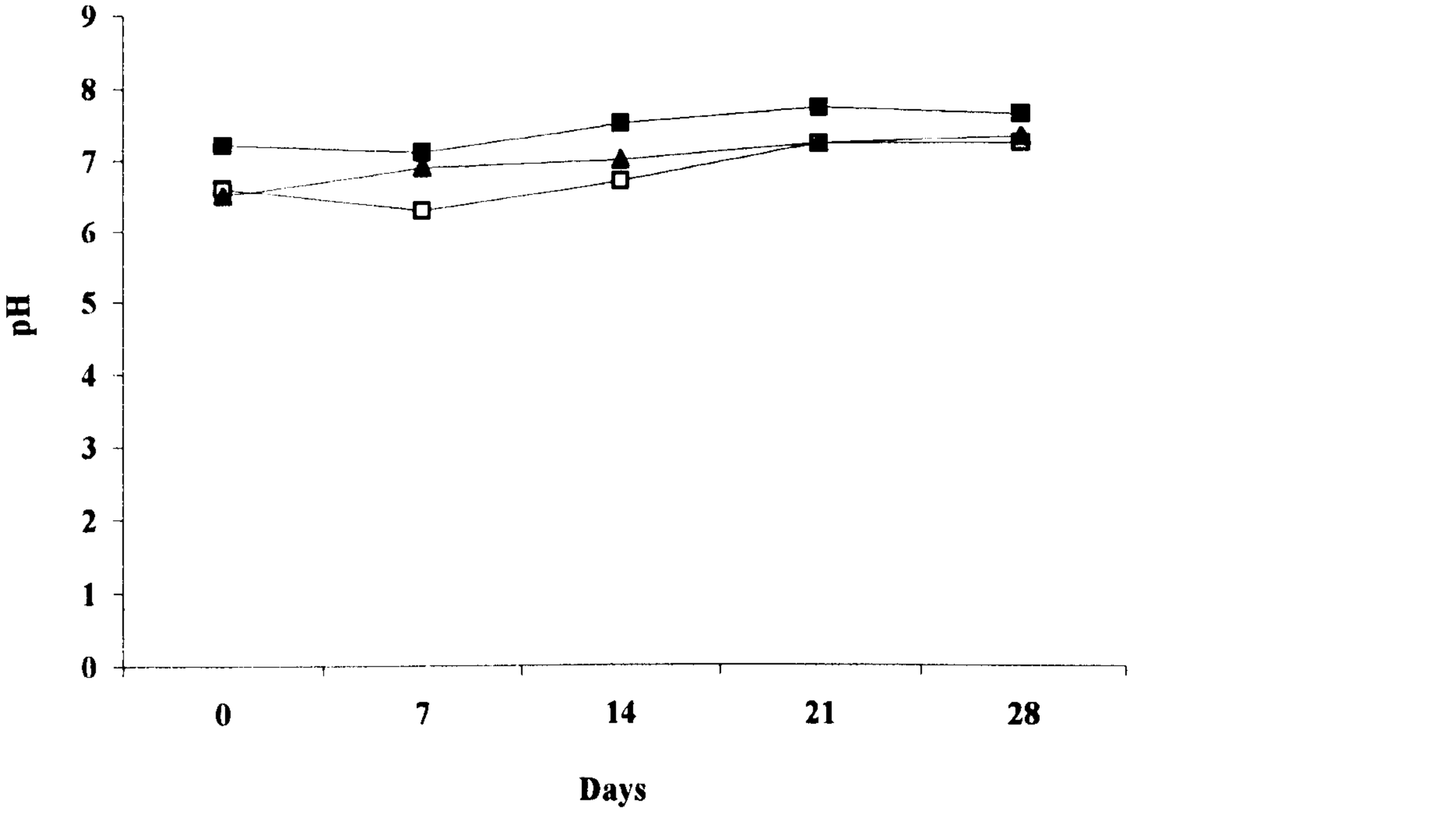

Agricultural soil
pH
9
8
7
6
5
4
3
2
1
0
0
7
14
21
28
Days

FIG: 2.7

Effect of added lime on the release of soluble silicon from deciduous and coniferous soils, (amended with silicic acid, sodium silicate and rock potash), under aerobic conditions.

(a) Deciduous woodland soil (under beech bulk *Fagus*)

(b) Coniferous soil (under *Pinus pine*)

—□— Control containing lime (soil lacking added silicon)

—▲— Soil amended with silicic acid and lime

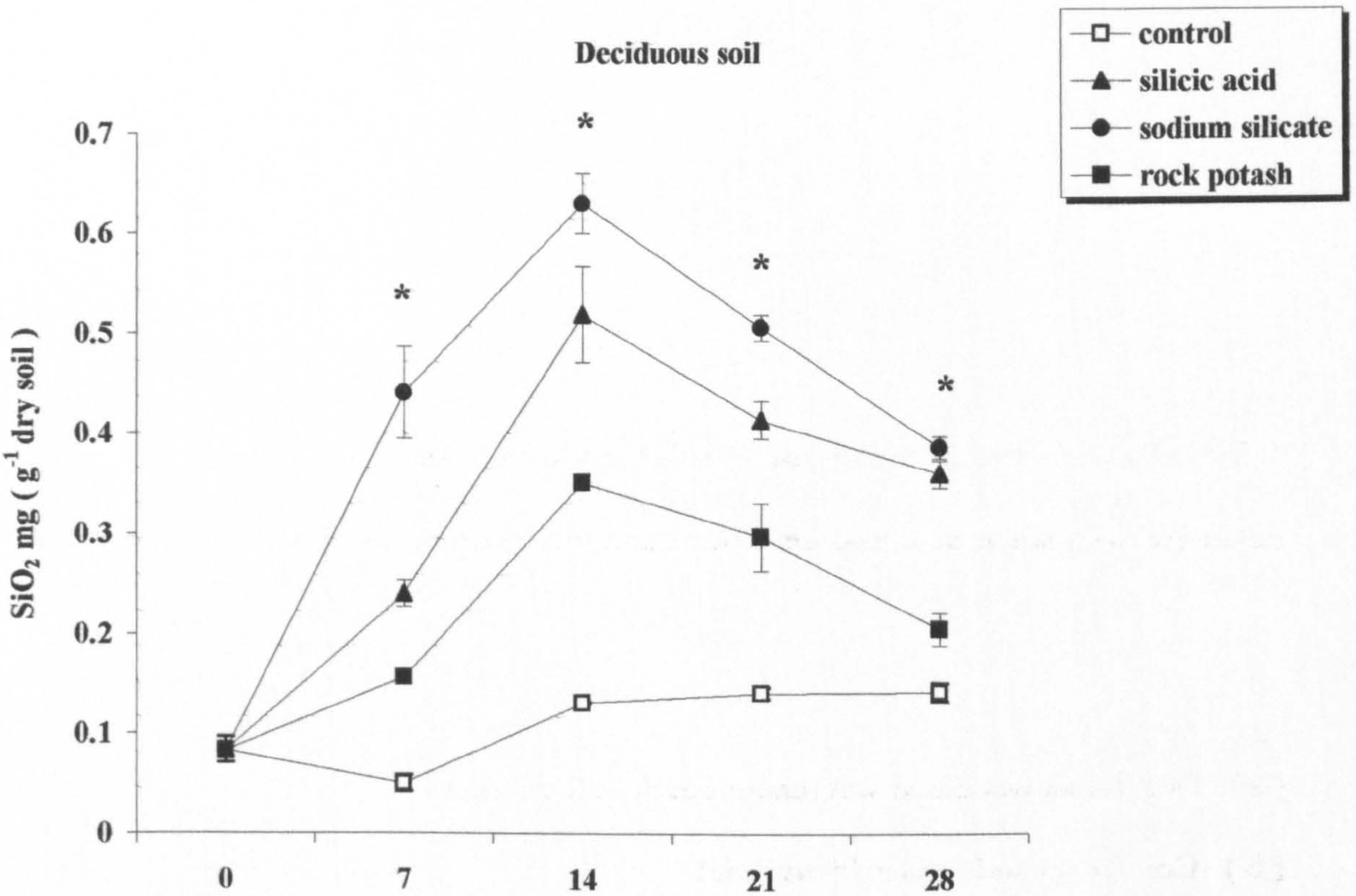
—●— Soil amended with sodium silicate and lime.

—■—Soil amended with rock potash and lime.

Means of triplicate, ± standard error. *Significant difference from control value, $P<0.05$.

FIG: 2.7

(a)


Deciduous soil
control
silicic acid
sodium silicate
rock potash
SiO_2 mg (g^{-1} dry soil)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
7
14
21
28
*
*
*
*

(b)

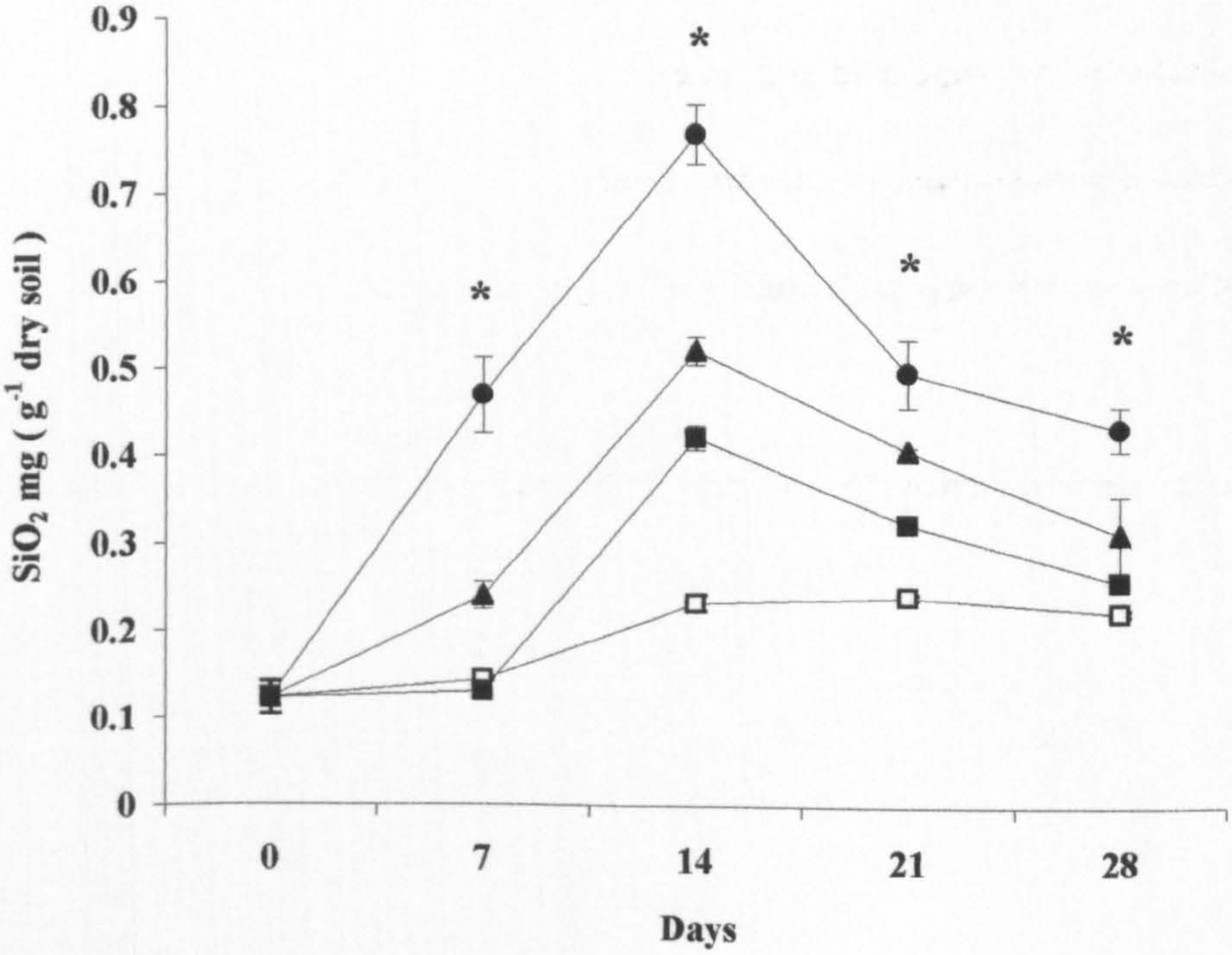

Coniferous soil
SiO_2 mg (g^{-1} dry soil)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
7
14
21
28
Days
*
*
*
*

FIG: 2.7

Effect of added lime on the release of soluble silicon from fern and agricultural soils (amended with silicic acid, sodium silicate and rock potash), under aerobic conditions.

(c) Fern soil (*Pteridium aquilinum*)

(d) Agricultural loam soil

—□— Control containing lime (soil lacking added silicon)

—▲— Soil amended with silicic acid and lime

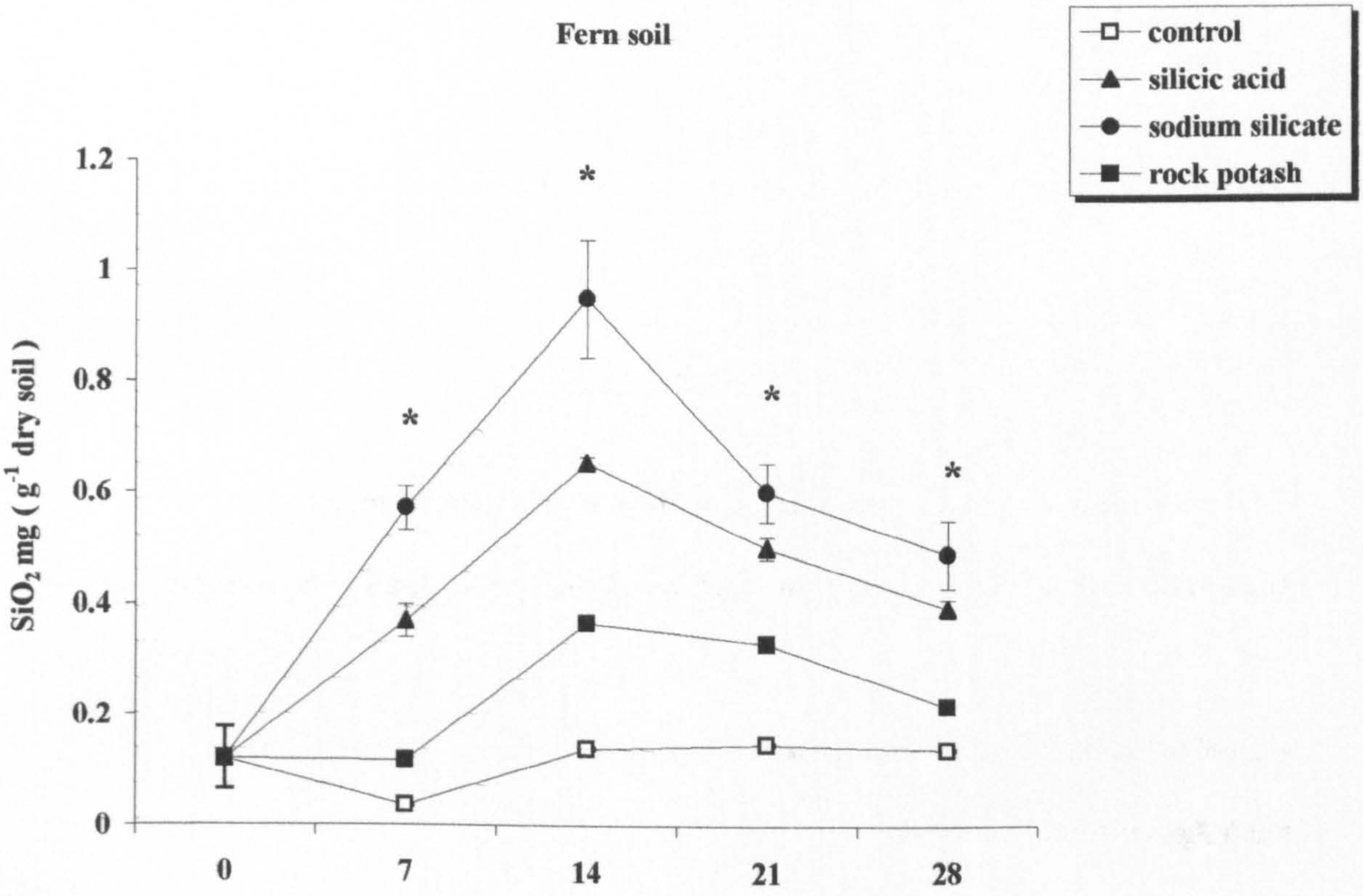
—●— Soil amended with sodium silicate and lime.

—■— Soil amended with rock potash and lime.

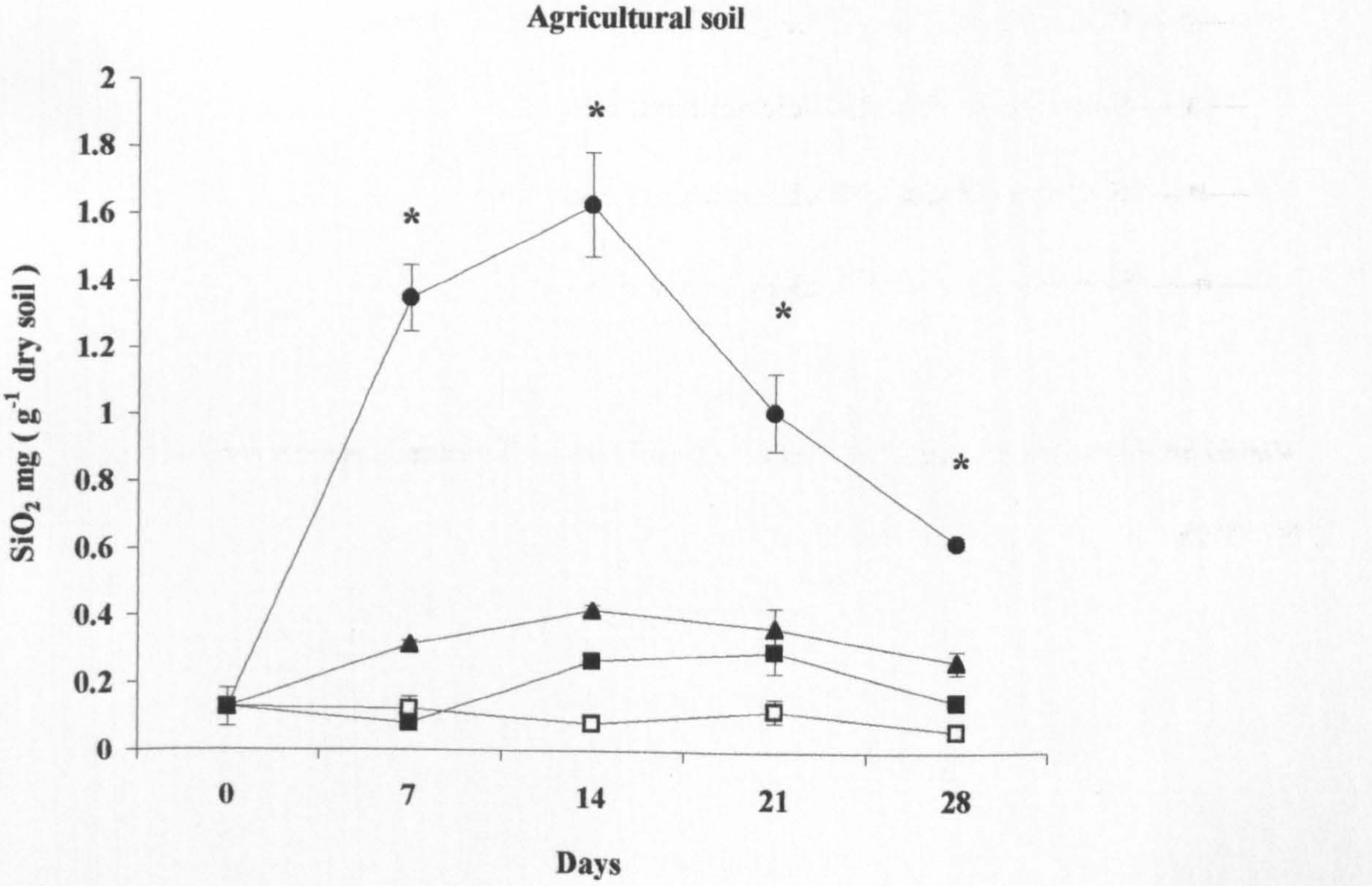
Means of triplicate, ± standard error. *Significant difference from control value, $P < 0.05$.

FIG: 2.7

(c)


Fern soil
control
silicic acid
sodium silicate
rock potash
1.2
1
0.8
0.6
0.4
0.2
0
SiO_2 mg (g^{-1} dry soil)
*
*
*
0
7
14
21
28

(d)


Agricultural soil
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
SiO_2 mg (g^{-1} dry soil)
*
*
*
*
0
7
14
21
28
Days

FIG; 2.8

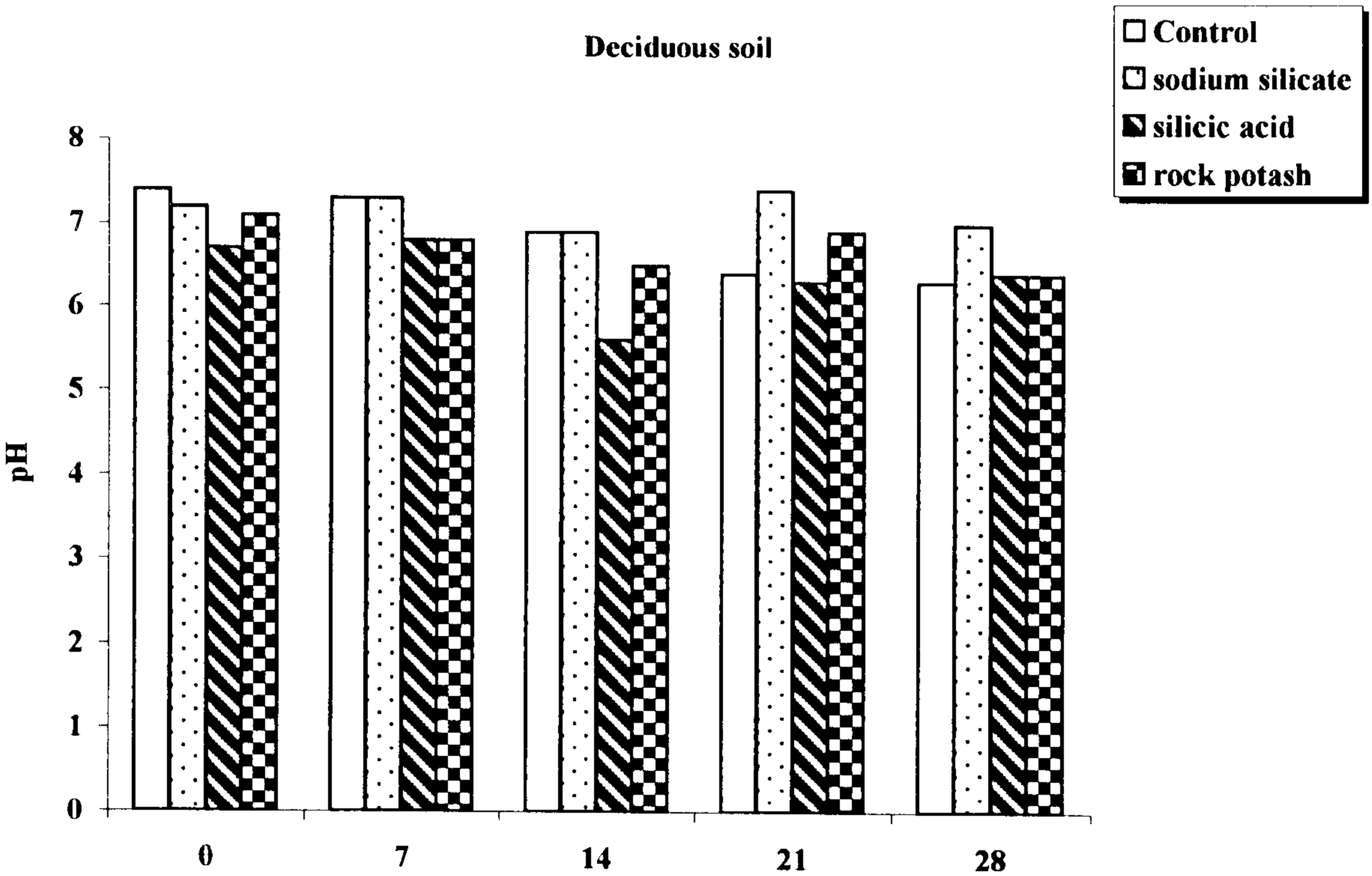
Effect of added sodium silicate, silicic acid and rock potash on soil pH

(a) Deciduous soil

(b) Coniferous soil

FIG: 2.8

(a)


Deciduous soil
Control
sodium silicate
silicic acid
rock potash
pH
8
7
6
5
4
3
2
1
0
0
7
14
21
28

(b)

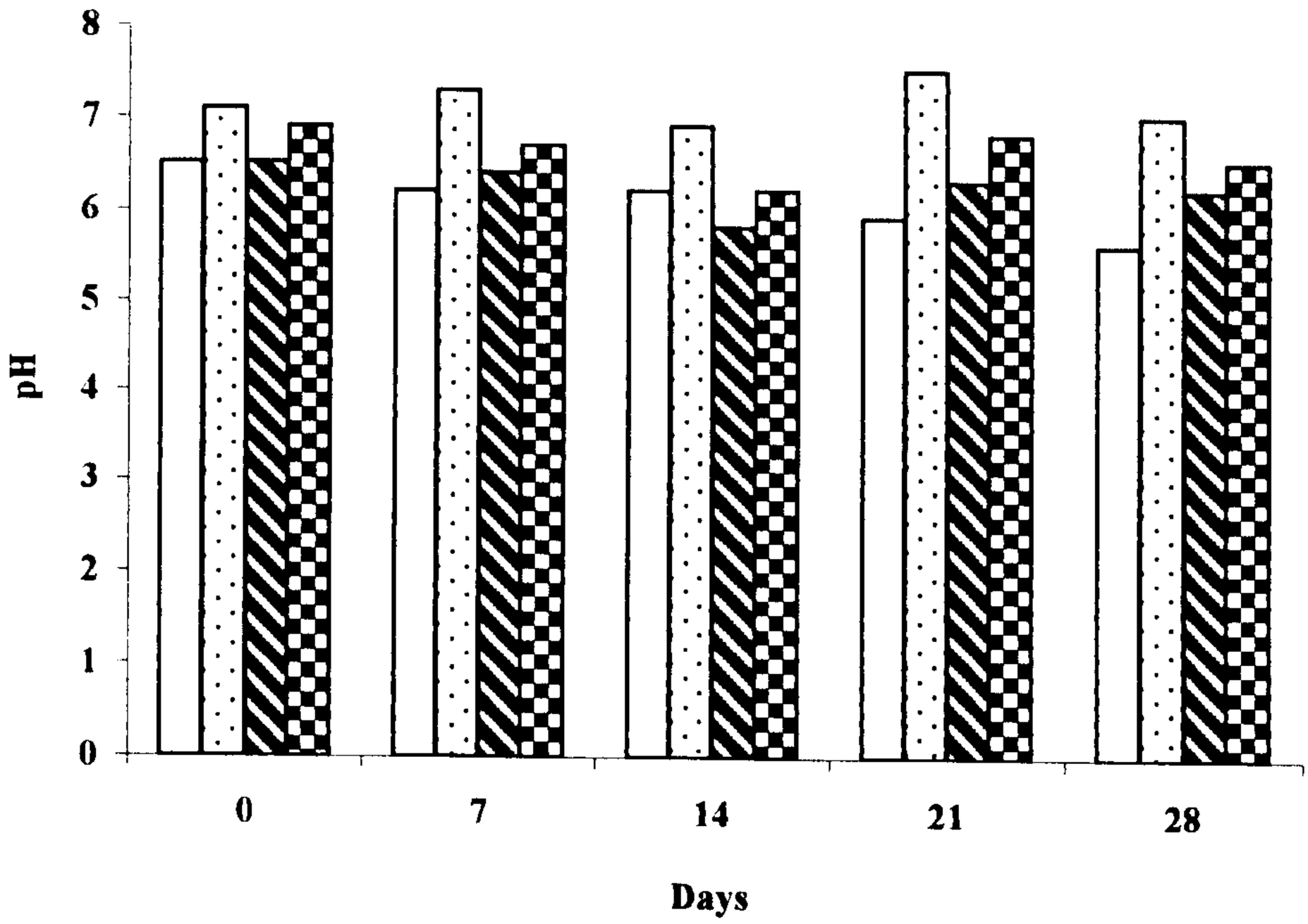

Coniferous soil
pH
8
7
6
5
4
3
2
1
0
0
7
14
21
28
Days

FIG: 2.8

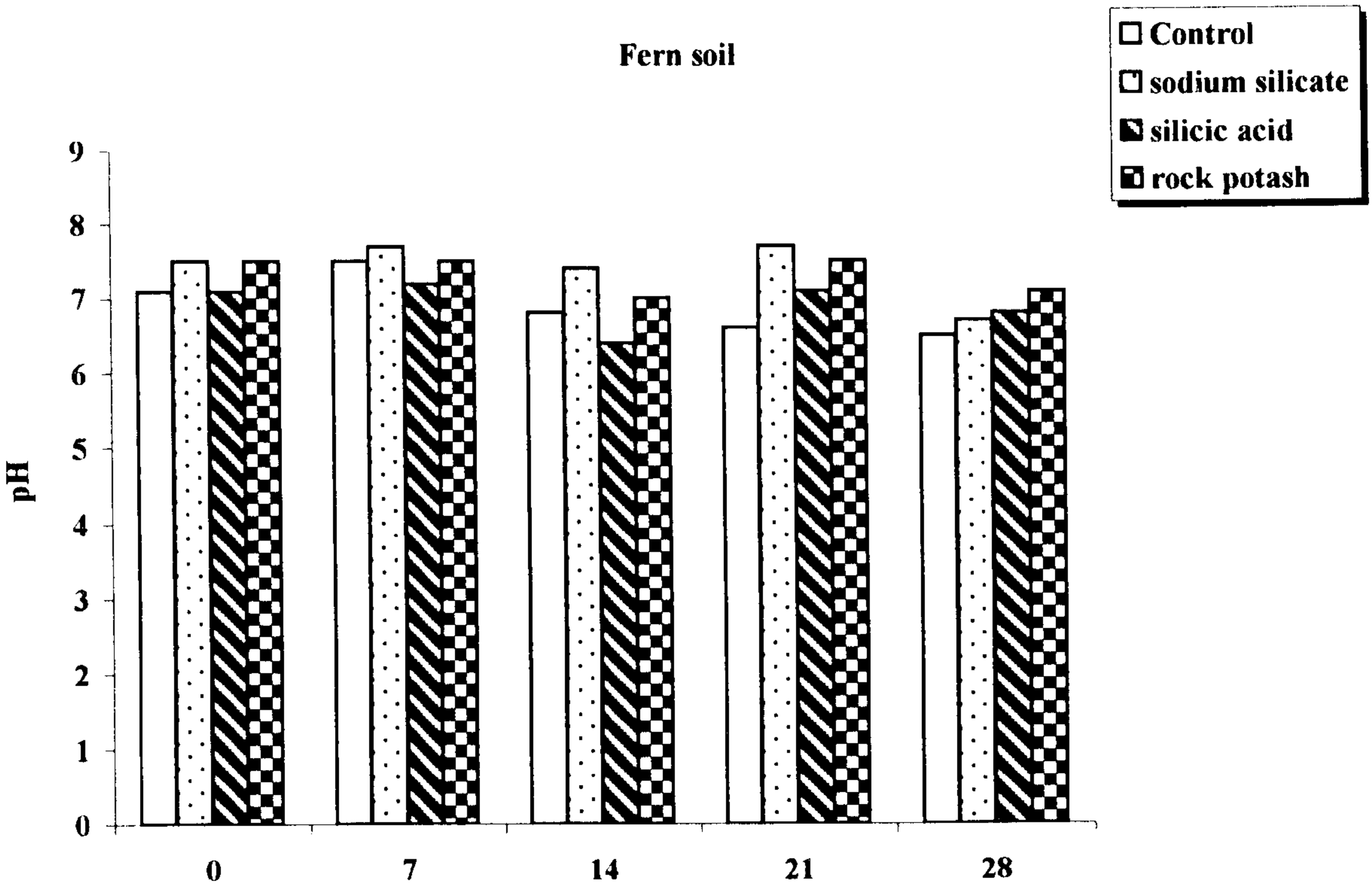
Effect of added sodium silicate, silicic acid and rock potash on soil pH.

(c) Fern soil

(d) Agricultural soil.

FIG: 2.8

(c)


Fern soil
Control
sodium silicate
silicic acid
rock potash
pH
9
8
7
6
5
4
3
2
1
0
0
7
14
21
28

(d)

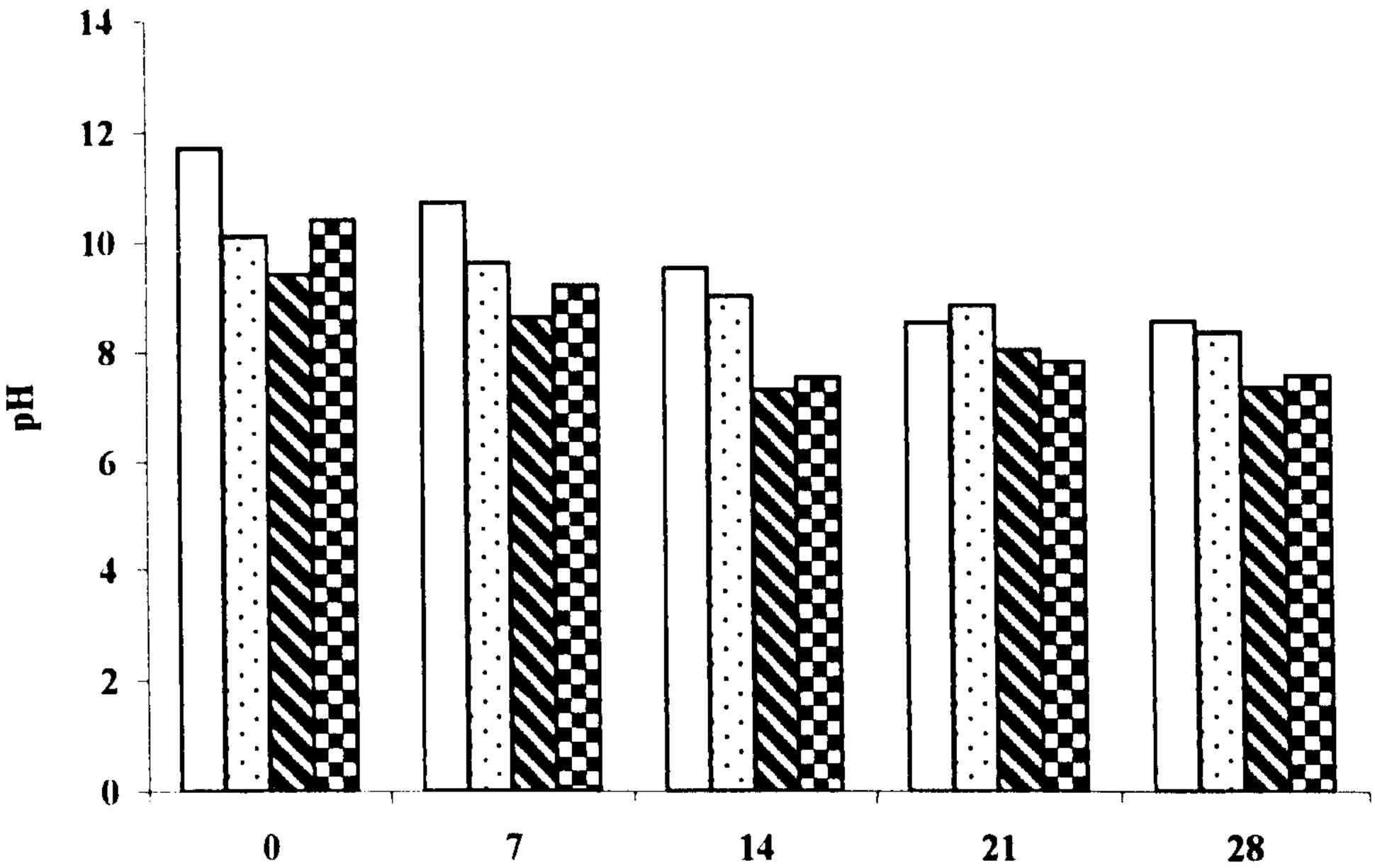

Agricultural soil
pH
14
12
10
8
6
4
2
0
0
7
14
21
28
Days

FIG: 2.9

Effect of temperature on the concentration of soluble silicon in agricultural soil, amended with silicic acid, sodium silicate and rock potash.

(a) Silicic acid.

(b) Sodium silicate

(c) Rock potash.

—□— Control (soil lacking added silicon)

—■— Treatment (soil amended with silicon)

Means of triplicate, ± standard error. *Significant difference from control value, P< 0.05.

FIG: 2.9

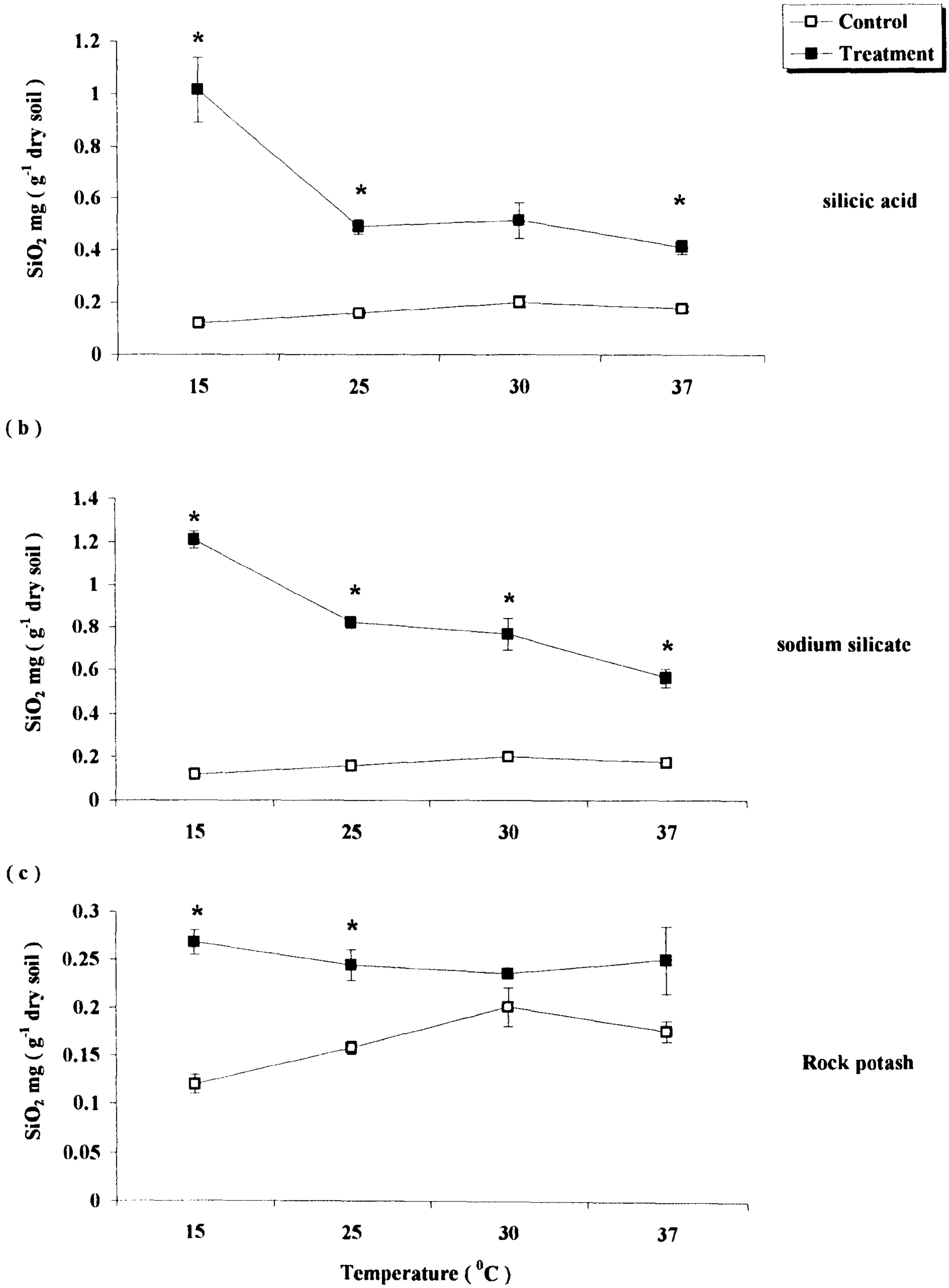

(a)
Control
Treatment
silicic acid
SiO_2 mg (g^{-1} dry soil)
1.2
1
0.8
0.6
0.4
0.2
0
*
15
25
30
37
(b)
sodium silicate
SiO_2 mg (g^{-1} dry soil)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
*
15
25
30
37
(c)
Rock potash
SiO_2 mg (g^{-1} dry soil)
0.3
0.25
0.2
0.15
0.1
0.05
0
*
15
25
30
37
Temperature (^{0}C)

FIG: 2.10

Effect of added silicon compounds on the pH of agricultural soil, at different temperatures.

(a) sodium silicate

(b) silicic acid

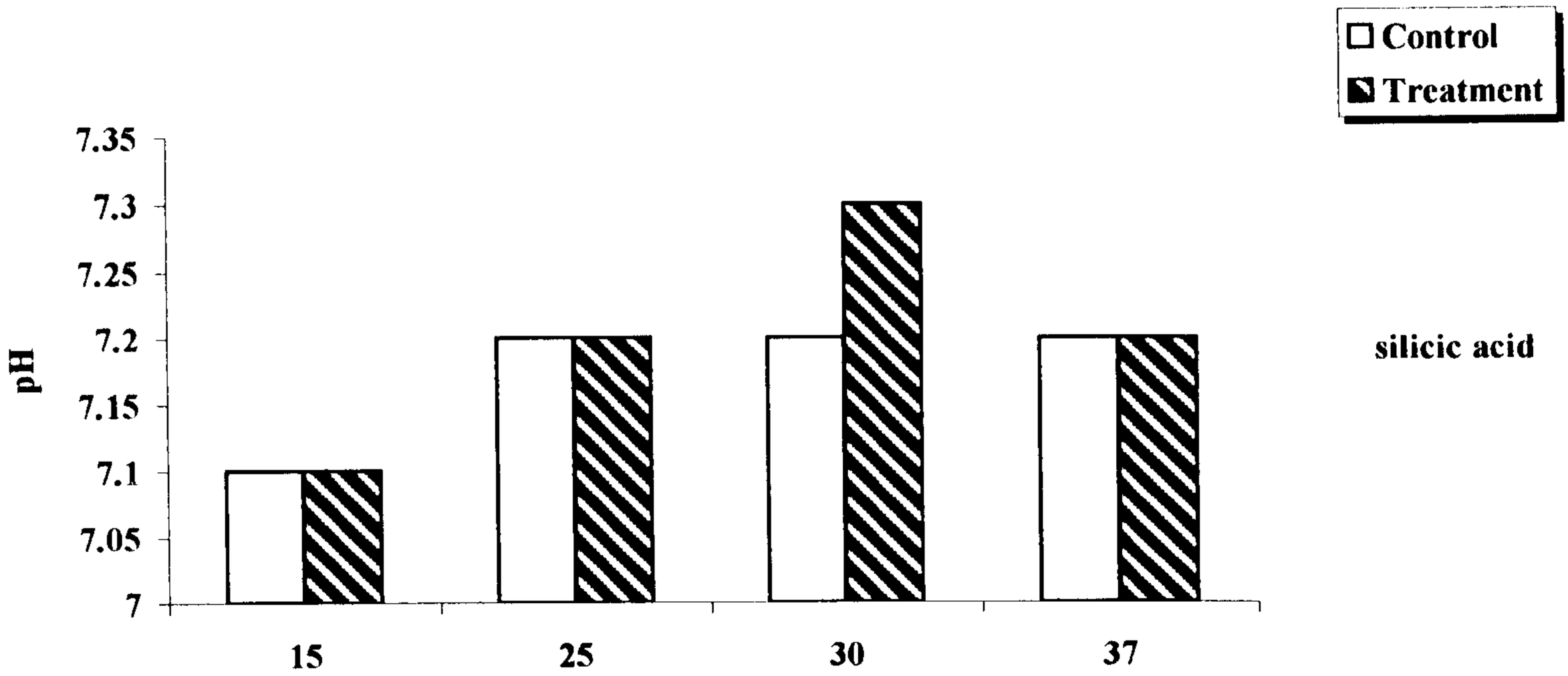
(c) rock potash

Control (lacking added silicon)

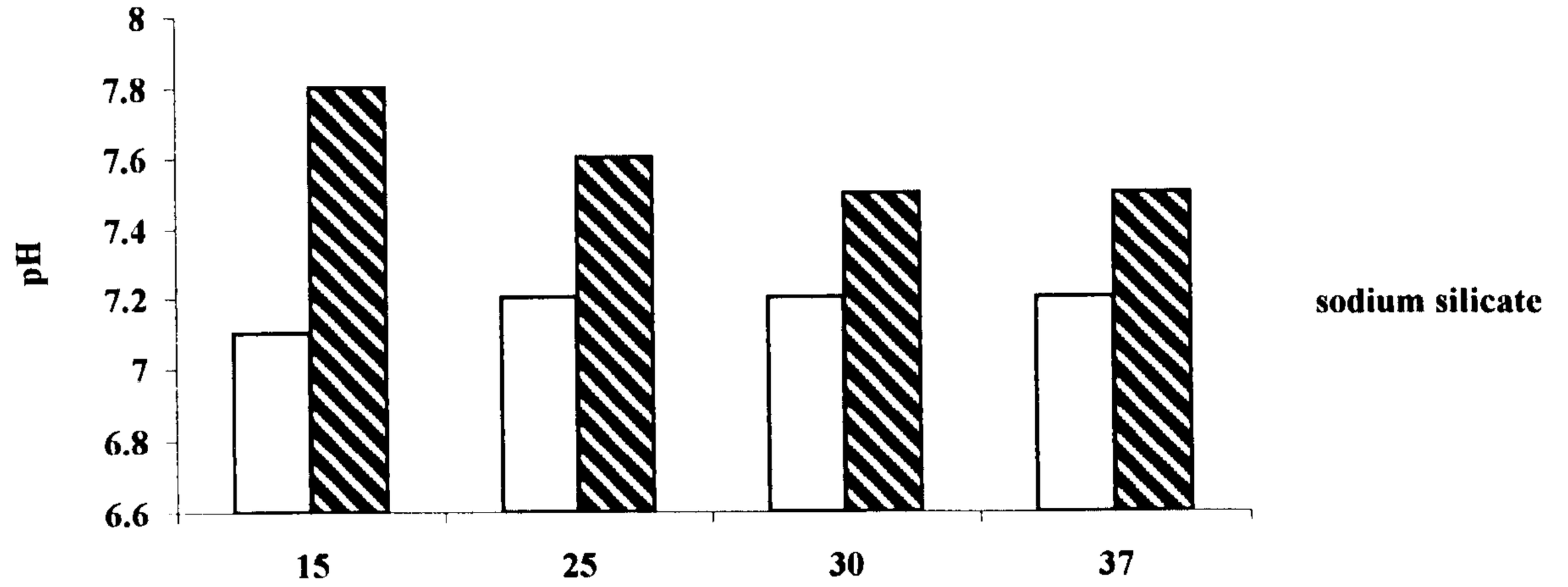
Treatment (soil amended with silicon)

FIG: 2.10

(a)


Control
Treatment
pH
7.35
7.3
7.25
7.2
7.15
7.1
7.05
7
15
25
30
37
silicic acid

(b)


pH
8
7.8
7.6
7.4
7.2
7
6.8
6.6
15
25
30
37
sodium silicate

(c)

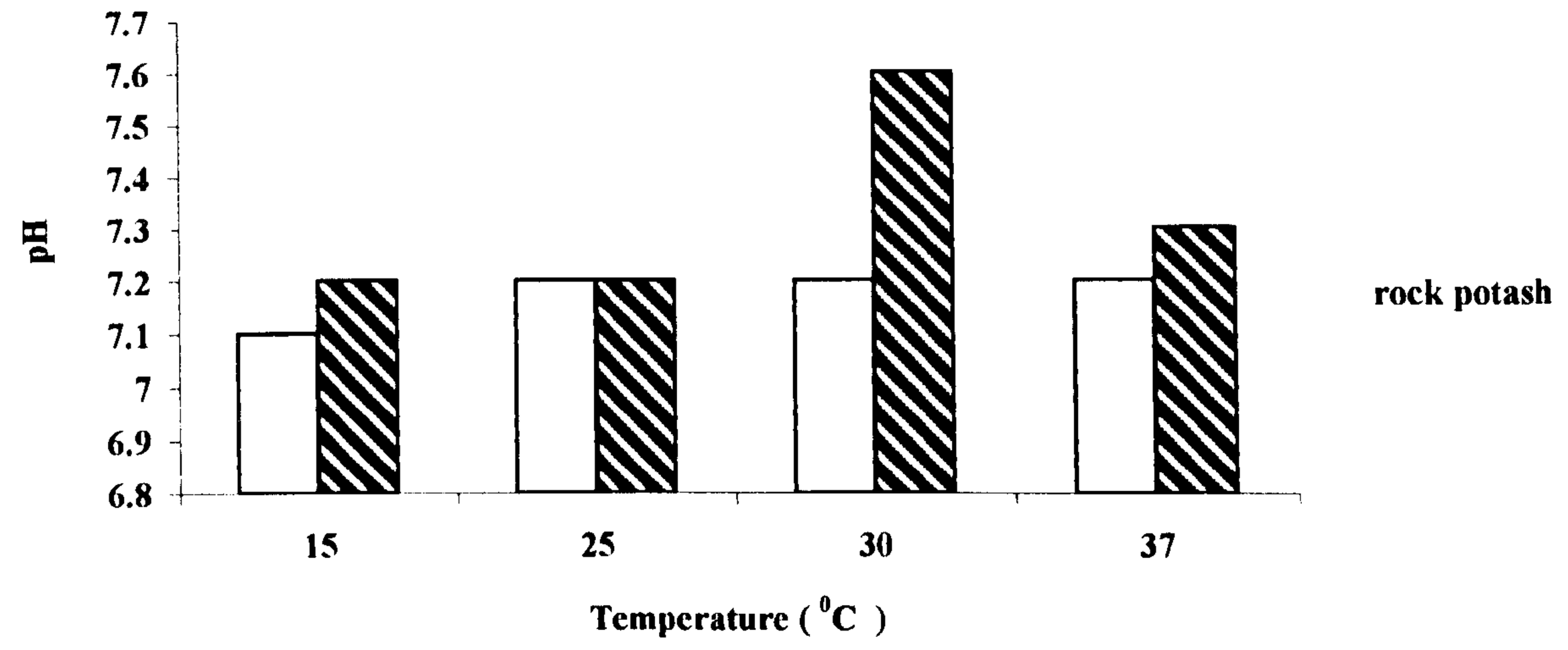

pH
7.7
7.6
7.5
7.4
7.3
7.2
7.1
7
6.9
6.8
15
25
30
37
rock potash
Temperature (^{0}C)

FIG: 2.11

Effect of added silicic acid on numbers of bacteria, isolated from deciduous and coniferous soils.

(a) Deciduous soil

(b) Coniferous soil

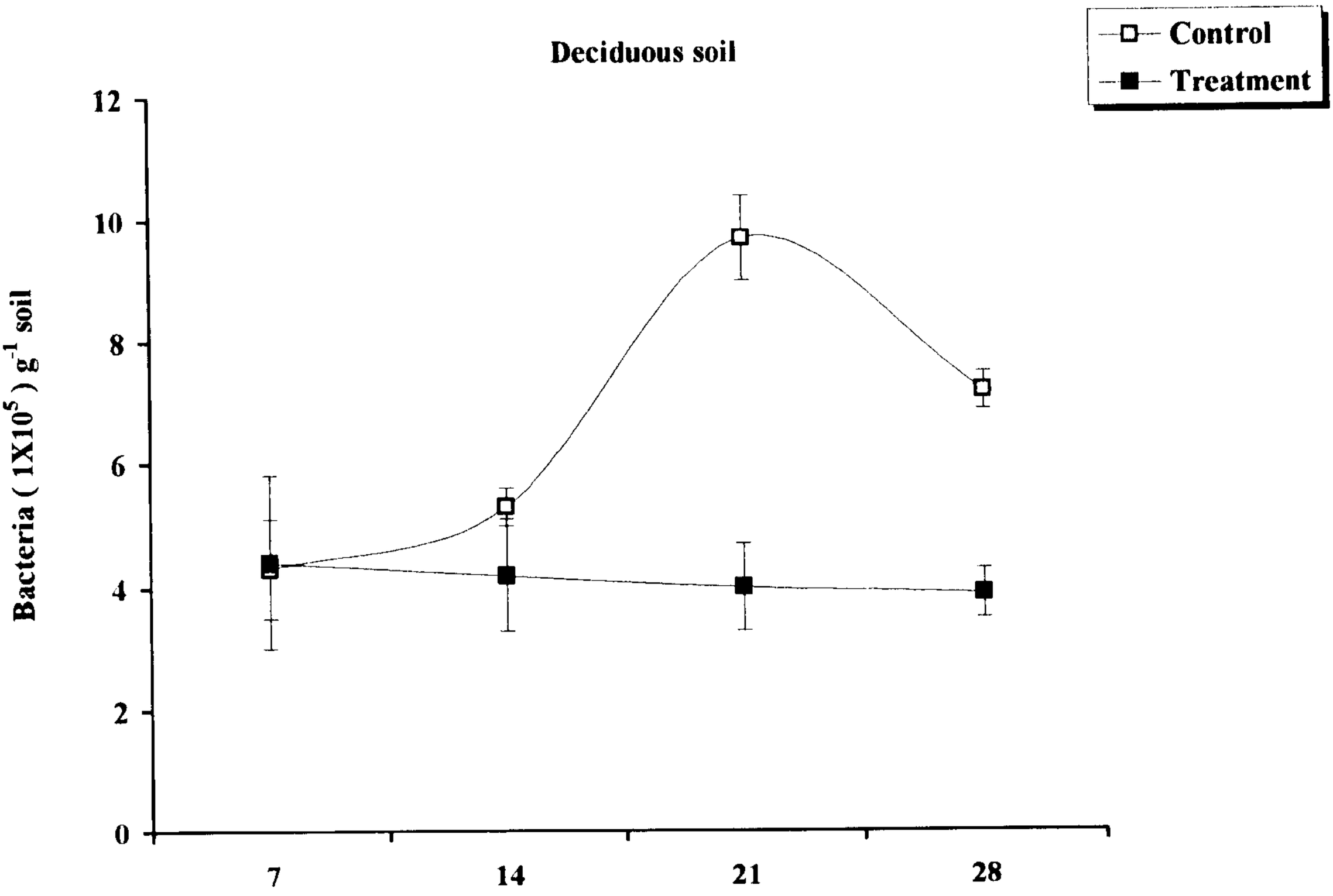
—□— Control (soil lacking added silicon)

—■— Treatment (soil amended with silicon)

Means of triplicate, ± standard error.

FIG: 2.11

(a)


Deciduous soil
Control
Treatment
Bacteria (1X10^5) g^{-1} soil
12
10
8
6
4
2
0
7
14
21
28

(b)

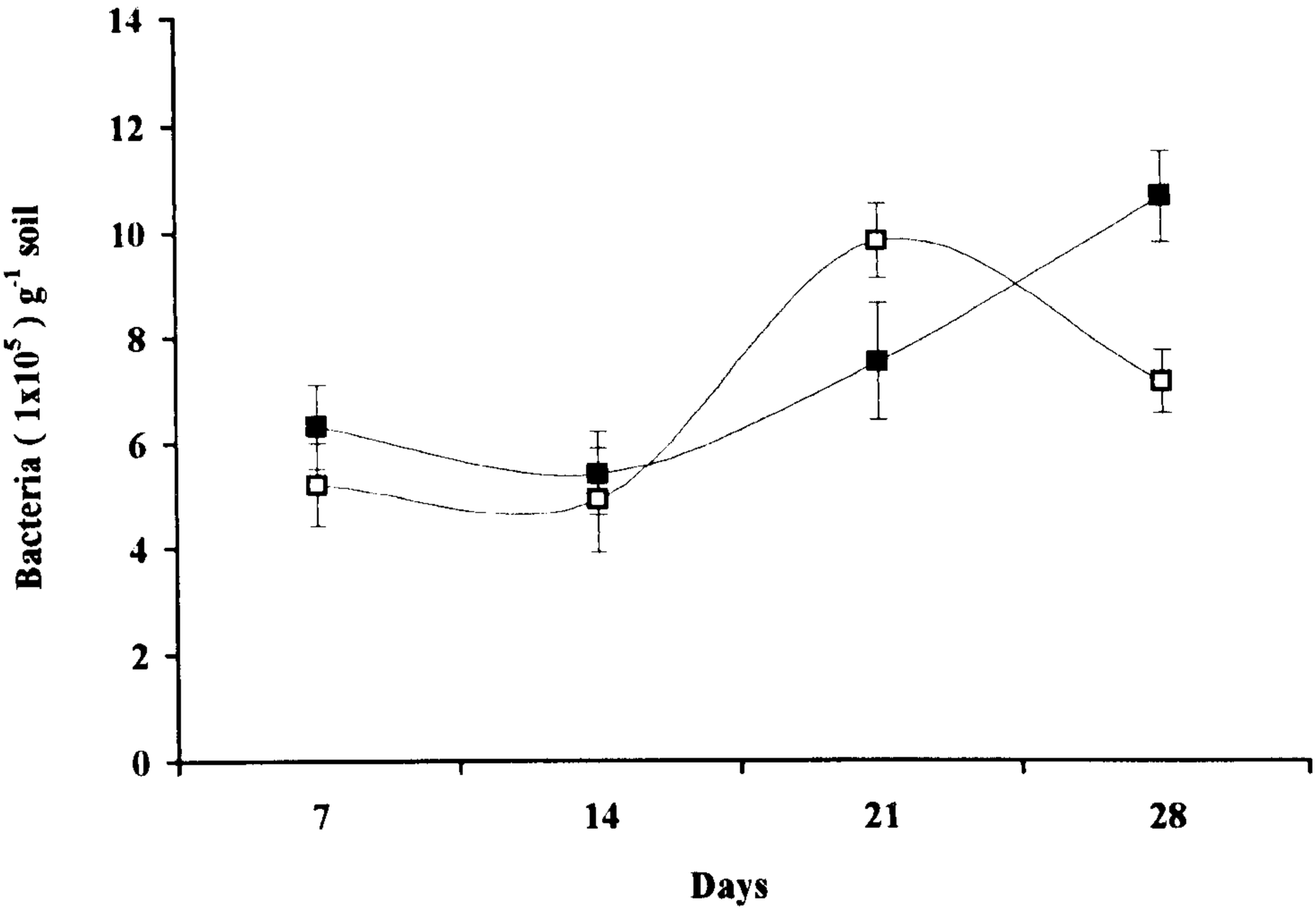

Coniferous soil
Bacteria (1x10^5) g^{-1} soil
14
12
10
8
6
4
2
0
7
14
21
28
Days

FIG: 2.11

Effect of added silicic acid on numbers of bacteria, isolated from fern and agricultural soils.

(c) Fern soil.

(d) Agricultural soil.

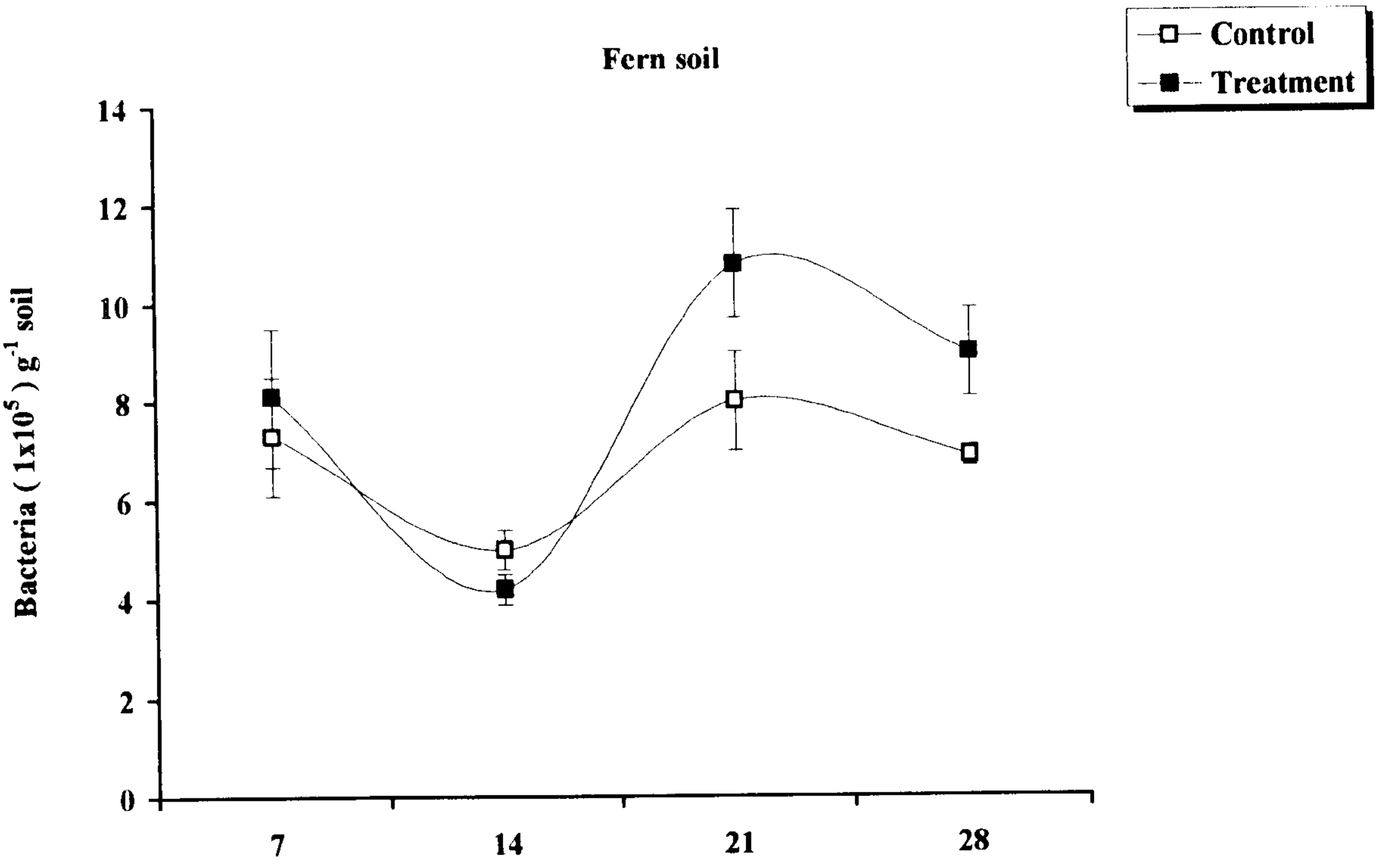
—□— Control (soil lacking added silicic acid)

—■— Treatment (silicic acid amended soil)

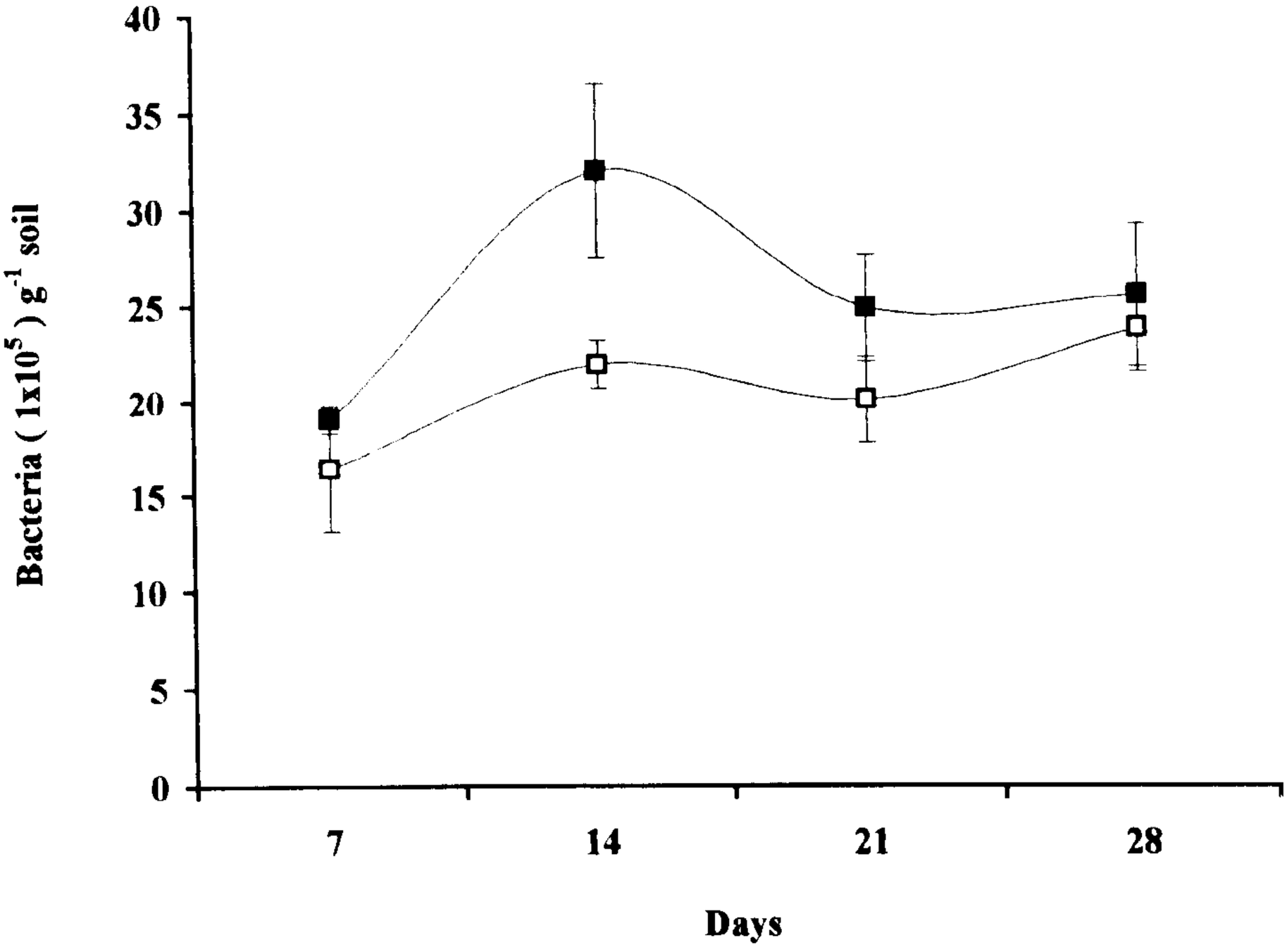
Means of triplicate, ± standard error.

FIG: 2.11

(c)


Fern soil
Control
Treatment
Bacteria (1x10^5) g^{-1} soil
14
12
10
8
6
4
2
0
7
14
21
28

(d)


Agricultural soil
Bacteria (1x10^5) g^{-1} soil
40
35
30
25
20
15
10
5
0
7
14
21
28
Days

CHAPTER THREE

EFFECTS OF SILICON ON NITRIFICATION IN AGRICULTURE SOIL

CHAPTER 3- EFFECTS OF SILICON ON NITRIFICATION IN AGRICULTURE SOIL

****

3.1 INTRODUCTION

3.1.1 Nitrogen cycle

Nitrogen (N) is an essential mineral nutrient for the growth of plants, animals, and microbes. The element is more susceptible to microbial transformations than phosphorus and potassium in soil. Nitrogen is a major building block for the synthesis of cell peptides and protein molecules, which are the basic components of life. Nitrogen occurs in microbial cell walls particularly chitin and peptidoglycans, enzymes and the nucleic acids.

Almost all of the nitrogen found in surface soil horizons is organic, in the form of proteins, chitin, urea, amino sugars etc. Viets, (1965) mentions that plants contain more nitrogen than any other element with the exception of hydrogen. Roots take up most of the plant's nitrogen requirements in the form of mineral nitrogen, largely ammonium and nitrate. Nitrate most often reaches the root by mass flow and ammonium by diffusion (Killham, 1994). Although the feature of mycorrhizal roots is that they are able to also take up a wide range of organic nitrogen compounds, particularly amino acids.

Most soils are deficient in nitrogen supply due mainly to insufficient mineralisation, for example NO_3^- is soluble in water, therefore unavailable by leaching and water transport and NH_4^+-NH_3 by volatilisation and via fixation, both by negative charged clay particles

and by soil organic matter. A shortage of nitrogen supply, badly affects soil fertility and crop-yield, resulting in yellow leaves and retarded growth.

Black, (1968) reported that it is not surprising that the growth of agricultural plants is limited more often by a deficiency of nitrogen than by other element. On the other hand excess nitrogen in soil may lead to a weakening of stems and to subsequent lodging of grains as well as to a reduction in fruit quality. Brady (1974) mentioned that excess nitrogen may reduce resistance to some plant diseases.

Löhnis (1913), formulated the concept of the nitrogen cycle, following identification of the forms of N in soil and the role of micro-organisms in moving N_2 from one form to another. He represented N as central to the cycle and recognized the involvement of protein, amide, NH_3, NO_2^-, and NO_3^- forms (Paul, 1996).

Nitrogen undergoes a number of transformations. The majority of those result from microbial activities, most often by bacteria, involving organic, inorganic and volatile compounds. This sequence of reactions results in the **nitrogen cycle** (Fig. 3.1). Cambell and Lees (1967), however, mentioned that there is "neither in the soil nor anywhere else, a nitrogen cycle;" but for the sake of simplicity a cycle in which the form of the element is constantly altered by the activities of micro-organisms can be assumed.

THE SOIL NITROGEN CYCLE

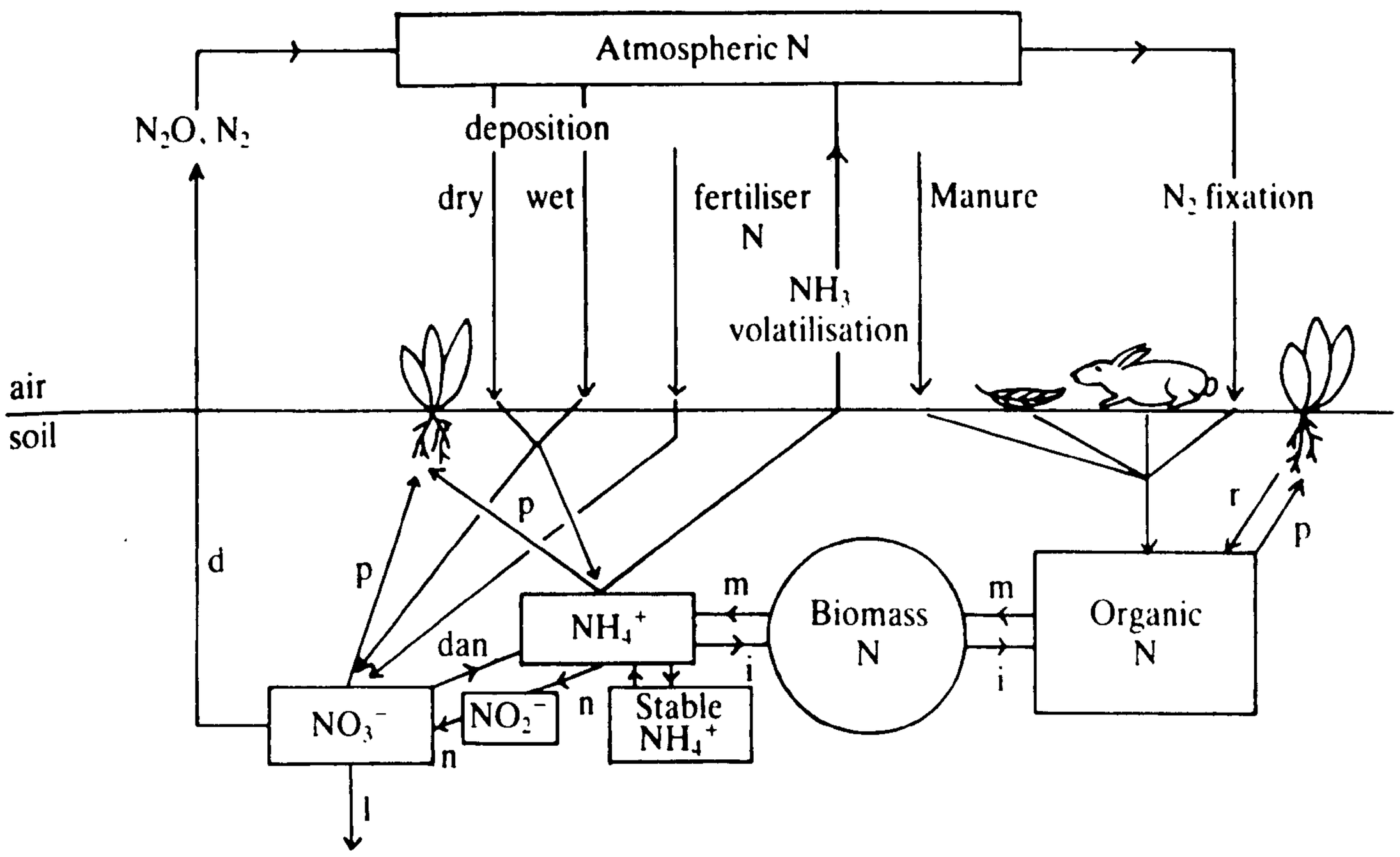


FIGURE 3.1 Abbreviations: d, denitrification; dan, dissimilatory and assimilatory nitrate reduction to ammonium; i, immobilization; m, mineralization; n, nitrification and subsequent leaching (l); p, plant uptake; r, root exudation and turnover.

(from Killham, 1994).

3.1.2 Atmospheric Nitrogen deposition into soil

Nitrogen, particularly from pollution sources, deposits from the atmosphere into the soil, often in bulk amounts in the form of nitric oxide (NO) and ammonia (NH_3), is known as "dry deposition" and in the form of nitrate (NO_3^-) and ammonium (NH_4^+) is called "wet deposition" (Killham, 1994).

The nitrogen cycle can be described briefly as follows:

a. Nitrogen fixation: The conversion of molecular nitrogen into nitrogenous compounds is termed *nitrogen fixation,* which is largely achieved by six main types of micro-organisms in the soil (a) free-living bacteria such as *Bacillus, Klebsiella* (facultative anaerobes), *Clostridium* (obligate anaerobe), (b) bacteria of the genus *Rhizobium,* (these fix N_2 in the root nodules of leguminous plants (c) actinomycetes of the genus *Frankia,* (these fix N_2 in the root nodules of non-leguminous plants), (d) free living cyanobacteria on the surface of soil (e.g. *Nostoc* and *Anabaena*); (e) symbiotic cyanobacteria (found in the lichen symbiosis) and (f) rhizocoenoses, N_2-bacteria loosely associated with the roots of some plants (the bacteria *Azotobacter, Beijerinckia* and *Azospirillum*) (Killham, 1994). These organisms convert a small amount of the large reservoir of atmospheric N_2 to the organic form. The non-biological fixation of ammonium by soils can also have a major influence on the availability of nitrogen.

b. Organic nitrogen formation: Nitrogen is then taken up by plants to synthesize proteins or nucleic acids. In turn, plants are consumed by animals and the N is converted to other simple and complex compounds.

c. Soil organic nitrogen: Animal excretion products, tissues of the dead animals and plants and dead micro-organisms are deposited in the soil. Micro-organisms use the resultant ammonia as a N source and to synthesize proteins.

d. Organic nitrogen degradation: The complete breakdown of proteins, nucleic acids and other compounds, by various micro-organisms yields a variety of amino acids.

e.Ammonification: The conversion of organic nitrogen to ammonium is termed as "ammonification". In this process, amino acids are deaminated by many non-specific micro-organisms (e.g. bacteria, actinomycetes and fungi), resulting in ammonia (NH_3) release, which is volatile, and if solubilized, ammonium (NH_4^+) is formed.

f. Nitrification: Ammonium (NH_4^+) ions are oxidised to nitrate (NO_3) through nitrite (NO_2) by nitrifying micro-organisms (particularly by *Nitrosomonas* and *Nitrobacter*). Three types of nitrification are involved: chemoautotrophic, heterotrophic, and methylotrophic nitrification.

g. Denitrification: This is the reduction of nitrate (NO_3^-) via intermediates to gaseous nitric oxide (NO), nitrous oxide (N_2O) and free nitrogen (N_2) gas, which can be lost to the atmosphere. This process is carried out by denitrifying micro-organisms particularly by species of *Pseudomonas*, *Bacillus* and *Paracoccus.* Denitrification is usually regarded as an anaerobic process (Tiedje *et al.*, 1981), although aerobic denitrification can occur (Robertson and Kuenen, 1985,b). Microbial reduction of nitrate takes place by two processes, one **assimilatory,** where the ion is reduced to nitrite and ammonium, this involves nitrate and nitrite reductases. The products of nitrate assimilation are incorporated into cell material. In nitrate assimilation, nitrogen therefore remains in the soil and remains potentially available as a plant nutrient. The second reduction process is known as **dissimilatory**, where nitrate acts as an alternative electron acceptor to oxygen in electron

transport chain. Dissimilatory nitrate reduction leads to the formation of either ammonium or dinitrogen; where dinitrogen is formed, the process is termed denitrification. Denitrification, by depleting part of the soil's reserve of an essential nutrients, has deleterious effects on crop production.

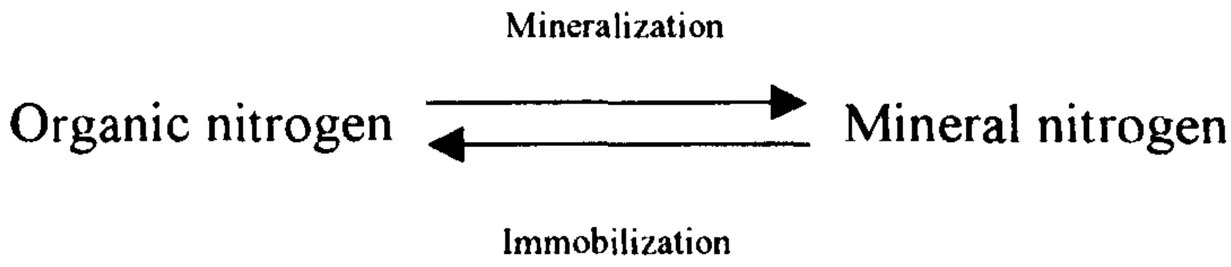
$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$$

3.1.3 Nitrogen mineralization

A process by which organic N_2 through the sequence of degradation of proteins, amino sugars, and nucleic acids, converts to a more mobile mineral form of nitrogen ammonium (NH_4^+).

3.1.4 Nitrogen immobilization

This is the reverse of mineralization, being the conversion of mineral nitrogen, back to the organic form of nitrogen in soil.



3.1.5 Proteolysis

Plants cannot utilize the nitrogen as a nutrient, where it is trapped in the form of proteins. In order to set free this organically bound nitrogen the first essential process by which the microbial enzymes "proteinases", hydrolyse the proteins is known as proteolysis. Here, proteins are converted to smaller units "peptides". Examples of some enzymes include serine proteinases, sulfhydryl proteinase of plant origin (e.g. papain) and an acid proteinase (pepsin). Peptides, are further divided into amino acids by the attack of "peptidases", elaborated by micro-organisms. Amino acids, thus liberated serve as both carbon and nitrogen sources for heterotrophs and higher plants. Further decomposition of amino acids to release carboxyl groups occurs by decarboxylation. Proteolytic enzymes are largely produced by some bacteria e.g. *Clostridium histolyticum, Cl. sporogenes,* and less actively by species of the genera *Proteus, Pseudomonas,* and *Bacillus*. Several fungi including *Alternaria, Aspergillus, Mucor, Penicillium,* and *Rhizopus,* and soil actinomycetes are also extremely proteolytic.

3.1.6 Nitrification

Nitrification is the biological formation of nitrate or nitrite from compounds containing reduced nitrogen (Alexander, 1977). Here the term "reduced" refers to the ammonium ion, which is oxidised to nitrite and nitrate, respectively. More recently, the nitrification has been defined as " the biological oxidation of any reduced form of nitrogen to a more oxidised form" (Killham, 1994). Nitrification is carried out in most of the soils, largely by some specific species of chemoautotrophic Gram-negative bacterial genera *Nitrosomonas* and *Nitrobacter.*

In the past, nitrification was believed to be beneficial to soil fertility and any limit to the process was thought as being detrimental to plant growth. Now, however, it is often regarded as undesirable because of the conversion of positively charged ammonium ions, which tend to be attracted and bound by negatively charged clay particles and organic matter in soil, to the readily leached nitrate state (Alexander, 1965). Nitrification occurs throughout the soil. It may not occur, however, in the rhizosphere due to allelopathy. Although allelopathy may occur, nitrifiers can be isolated from rhizosphere soil. Net nitrification may also often not occur because of uptake and immobilization of nitrate by both the plant root and rhizosphere heterotrophic micro-organisms. (Killham, 1994). Nitrification in soil is also affected by various environmental factors e.g. pH, oxygen supply, temperature, moisture regime, organic matter levels or supply, carbon dioxide content and cation exchange capacity of a soil (Mahendrappa *et al.*, 1966).

3.1.7 Effects of nitrification

Nitrification can create undesirable conditions.

a. Ammonium (cation) can be adsorbed to soil particles, and become relatively immobile.

b. Nitrate, (anion) is mobile in soil solution which can be leached away from the root zone particularly in sandy soils, under heavy rainfall, or where excess irrigation is practiced. Nitrate is also susceptible to losses through denitrification. Excess NO_3^- leached from soil often accumulates in ground water, streams and lakes, thereby producing excess plant and algal growth, (i.e. "eutrophication"). Nitrate also causes infant and animal methemoglobinaemia and form carcinogenic nitrosamines by reaction with other nitrogenous compounds.

3.1.8 The biological aspect of nitrification

The biological nature of nitrification was first illustrated by Schloesing and Muntz in 1877 (Russell, 1973), but the nitrifying bacteria were not isolated until 1889, when Winogradsky demonstrated their ability to grow autotrophically on a medium lacking organic carbon (Waksman, 1946). In recent years, our understanding of the ecology of soil nitrification has changed. The first development has been the realisation that nitrification is not as restricted by soil pH as was traditionally thought. The second aspect of this change in our understanding about nitrifier ecology is that two types of nitrification are now recognised:, chemoautotrophic and heterotrophic nitrification (Killham, 1994). It appears that chemoautotrophic nitrification dominates in agricultural soils, whereas a role for heterotrophs has been implicated in nitrification in acidic soils (Schmidt, 1982).

3.1.9 Chemoautotrophic nitrification

Chemoautotrophic nitrification is assumed to have carried out in most of the soils, by species of two predominant genera, *Nitrosomonas* and *Nitrobacter.*

Chemoautotrophic bacteria are classified into two groups,

1) The bacteria which derive energy for cell synthesis by the oxidation of ammonium.

2) The bacteria which derive energy from the oxidation of nitrite.

The nitrifying bacteria are gram negative, without endospores, aerobic, chemoautotrophs and exhibit a variety of shapes including rods, ellipsoids, cocci and spirilla (Alexander, 1977). These bacteria are typically obligate chemolithoautotrophs and hence rely upon the oxidation of reduced nitrogen for their energy, while synthesizing all of their cell constituents from carbon dioxide (Schmidt, 1982)

Meiklejohn, (1953; 1954) mentions that species of the genus *Nitrosomonas* are generally considered to be the most important group involved in the first oxidation; while species of *Nitrobacter* are important in the second. However, according to Belser and Schmidt (1978), a number of different genera of ammonium oxidising bacteria could be isolated from soils. The number of NH_4^+ oxidisers found to vary from zero to one million or more g^{-1} of soil. (Alexander, 1977); the highest counts being found at soil pH more than 6.0.

Ammonia oxidisers are classified on the basis of shape, membrane constituents, and G+C contents (Table 3.1). *Nitrosomonas* and *Nitrosospira,* have been identified as the most common NH_4^+ oxidisers in soil. (Paul, 1996). The ammonium oxidizers appear incapable of using organic compounds, even as partial energy sources, where as certain strains of nitrite oxidisers (*Nitrobacter*) can grow heterotrophically and are facultative rather than

obligate autotrophs (Smith and Hoare, 1968: Bock, 1976). They are, however capable of assimilating organic compounds such as yeast extract and amino acids which may prove growth stimulatory (Delwich, 1965; Clark, 1967).

Many scientists, in the past, thought that the nitrifying bacteria are obligate autotrophs, which obtain energy from the oxidation reactions occuring in nitrification. Carbon was considered to be derived from CO_2 (for cell synthesis), carbonates and bicarbonates, using the energy derived from oxidative reactions. Until recently these bacteria were thought to be incapable of utilizing organic nutrients.

Recently, Smith and Hoare (1968) found that *Nitrobacter agilis* could grow on acetate without nitrite or CO_2. This suggests that this bacterium can grow as a facultative autotroph. The ability to switch from CO_2 and inorganic N to acetate could confer advantages when growing in the environment, if the former were rate limiting, i.e. nitrifying bacteria can now be considered to be mixotrophs.

Schimidt (1982) indicated, these organisms are typically obligate chemolithoautotrophs, oxidising reduced nitrogen for their energy whilst synthesizing all of their cell constituents from carbon dioxide. In the ammonium oxidizing bacteria, ammonia rather than ammonium crosses the cytoplasmic membrane and is oxidised to hydroxylamine (Suzuki et al., 1974).

The chemical reactions carried out thus,

$$NH_4^+ + 1/2O_2 + H \rightarrow NH_2OH + H^+$$

The oxidation of hydroxylamine resulting in the production of nitrite and energy.

$$NH_2OH + O_2 \rightarrow HNO_2 + H_2O$$

The oxidation of nitrite to nitrate occurs by the addition of oxygen atom from water and not molecular oxygen.

$$NO_2^- + 1/2O_2 \rightarrow NO_3$$

TABLE (3.1) **CHEMOAUTOTROPHIC NITROGEN OXIDISERS**
(from Paul, 1996)

Genus	Species	Characteristics and habitats
Nitrosomonas	*europeae*	Oxidize NH_4^+ to NO_2^- Rod to ellipsoidal, intracytoplasmic membranes as flattened vesicles, G+C 51%. Soil, water, sewage.
Nitrosospira	*briensis*	Acid soils
Nitrosococcus	*nitrosus*	Spherical to ellipsoidal. Soil, marine.
	oceanus	Flattened membrane vesicles. Marine.
	mobilus	Spheres, additional cell wall layer. Marine.
Nitrosovibrio	*tenuis*	Slender curved rods, G+C 54%. Soil
Nitrosolobus	*multiformus*	Pleomorphic, lobate cells, central compartments surrounded by peripheral, G+C 56.4%. Soil.
Nitrobacter	*winogradskyi*	Rod to pear shaped, no separate peptido-glycon cell wall. Soil
	hamburgensis	
	vulgaris	Cytoplasmic inclusions, G+C 60-62%, grow heterotrophically, soil
Nitrococcus	*mobilus*	Marine isolate, spheres, tibular, cytomembrances, halophilic, G+C 61.2%. Marine.
Nitrospina	*gracilus*	Marine non-motile, rods, G+C 58%
Nitrospira	*marina*	Non-motile spirals, no intracytoplasmic membrane, G+C 50.5%

3.1.10 Heterotrophic nitrification

The involvement of heterotrophs in nitrification was first suggested in 1894. Only recently however, has heterotrophic nitrification been seriously considered as a soil process. A large number of heterotrophic nitrifiers have been found to be responsible for carrying out the oxidation of reduced forms of nitrogen. These micro-organisms include Gram negative, Gram-positive bacteria, spore and non-spore formers, anaerobes (Alexander, 1965), fungi (Eylar and Schmidt, 1959) and species of actinomycetes (e.g. *Nocardia corallina*) (Hirsch *et al.*, 1961). The heterotrophic bacteria include *Arthrobacter globiformis*, *Aerobacter aerogenes*, *Mycobacterium phlei*, *Streptomyces griseus*, *Thiosphaera*, and *Pseudomonas* spp. and the major organisms involved appear to be fungi as *Aspergillus flavus*, (first isolated as a nitrifier in 1954) and spp. of *Penicillium* and *Cephalosporium* (Paul, 1996). Heterotrophic nitrification by fungi is well documented (Schmidt, 1982; Killham, 1986). Fungi have also been reported to be able to produce substantial amounts of nitrate by nitrification, and are considered to be the most numerous and efficient of the heterotrophic nitrifiers (Odu and Adeoye, 1970).

Fungal nitrification is likely to be less limited by acidic conditions and should be more resistant to drought stress and alleleopathic chemicals than would autotrophic bacteria. (Paul, 1996). Aleem (1975) mentioned that the biochemistry of fungal nitrification has not been fully elucidated and that it is unclear whether fungi nitrify using an inorganic pathway, with hydroxylamine and nitrite as intermediates, or else use an organic pathway involving the oxidation of an amino or amide to a substituted hydroxylamine followed by oxidation to a nitrose and then to a nitro-compound (Doxtader, 1965). Heterotrophic nitrifiers are now

known to be capable of producing NO_3^- from both inorganic and organic sources (Paul, 1996). (Table 3.2)

TABLE (3.2) NITROGENOUS SUBSTRATES AND PRODUCTS OF SOME HETEROTROPHIC NITRIFYING MICRO-ORGANISMS.

(from Alexander, 1965).

Micro-organisms	Substrate	Product
	Bacteria	
Agrobacterium spp.	Pyruvic oxime	nitrite
Azotobacter chroococcum	NH_4	bound NH_2OH
Bacillus sp.	NH_4	nitrite
Clostridium butyricum	N_2	nitrate
Corynebacterium simplex	nitrophenols	nitrite
Mycobacterium rubrum	NH_2	nitrite
Pseudomonas spp.	NH_2OH	nitrite
Pseudomonas methanica	NH_4	nitrite
	Actinomycetes	
Micromonospora spp	NH_4	nitrite
Nocardia spp.	NH_4	nitrite
Nocardia corallina	pyruvic oxime	nitrite
Nocardia sp.	P-nitrobenzoate	nitrite
Streptomyces spp.	NH_4	nitrite
	Fungi	
Aspergillus flavus	NH_4	bound NH_2OH, nitrite β–nitropro-ionate, nitrate.
Aspergillus flavus	amino	aspergillus acid
Aspergillus niger	NH_4	NH_2OH
Aspergillus wentii	nitrite	nitrate
Cephalosporium sp.	NH_4	nitrate
Penicillium atrrovenetum β–nitropropionate	NH_4	
Penicillium spp.	nitrite	nitrate
Penicillium spp. hydroxy-	amino	N-formyl aminoacetate
Sterigmatocystis nigra	NH_4	NH_2OH

3.1.11 Methylotrophic nitrification

Methylotrophic nitrification, is a well established process which is carried out by methane oxidizing bacteria, capable of oxidising ammonium to nitrite and at a rate comparable to other heterotrophic nitrifiers (Whittenbury *et al.*, 1970; Verstraete, 1981). Methylotrophic nitrification is of recent interest since, like *Nitrosomonas* but in contrast to other nitrifying heterotrophs, the methylotrophs appear to be able to generate NADH from the oxidation of NH_2OH (Dalton, 1977). This suggests that their nitrification capacity might be directly linked to their energy metabolism. Methylotrophs have a low tolerance to ammonium i.e. only 200 mg NH_4^+-N l^{-1} compared to levels of 3000 mg NH_4^+-N l^{-1} tolerated by autotrophic nitrifiers (Verstraete, 1981). It is therefore unlikely that they will compete successfully with the autotrophic nitrifiers for ammonium oxidation. However, reports on methylotrophic nitrification in acidic soils have also appeared where autotrophic nitrification is inhibited (Verstraete, 1981; Kreitinger *et al.*, 1985).

The aim of the work presented in this Chapter was to determine the effects of silicon compounds added to different soils and to study these effects on ammonification and nitrification processes.

3.2 MATERIALS AND METHODS

3.2.1 Effect of sodium silicate, silicic acid and rock potash on nitrification in agricultural soil

To triplicate samples of agriculture soil (100 g; sieved <4mm and dried overnight at room temperature), in polythene bags, was added 10 ml (100 μg ml^{-1} NH_4^+-N), plus 1 g of the silicon compound, were added and mixed thoroughly. Controls were set-up lacking added silicon. All the samples were incubated at 25^0C. After incubation, 1g soil was added to 20 ml KCl (1.5 M), for ammonium-N and 20 ml deionised water (autoclaved), for the extraction of nitrite-N and nitrate-N ions, in screw capped glass bottle (150 ml). The contents were shaken for 15 minutes at 70 rpm using orbital shaker and filtered through Whatman No.1 filter paper. Fresh extracts were analysed every week, for the determination of ammonium-N, nitrite-N and nitrate-N by spectrophotometer. (see section 3.2.3)

3.2.2 Effect of different amounts of sodium silicate, silicic acid and rock potash on nitrification in agricultural soil

To agricultural soil (100 g; sieved <4mm and dried overnight at room temperature), in polythene bags, were added 10 ml (100 μg ml^{-1} NH_4^+-N), plus 1g, 5g, 10g and 20g, each silicon compound per soil bag in triplicates, were added and mixed thoroughly. Controls were set-up lacking added silicon. All samples were incubated at 25^0C.

The N-ions were determined as described in section 3.2.3.

3.2.3 Analysis of inorganic N ions

(A) Indophenol blue method for the determination of ammonium-N (NH_4^+-N)

(Wainwright & Pugh 1973)

To filtrate (2 ml) was added, distilled water (7 ml), *phenolate reagent (5 ml), and sodium hypochlorite (5 ml) solution (0.9% v/v active chlorine), mixed and incubated at 25^{0}C for 20 minutes in the dark. The intensity of indophenol-blue-ammonium-complex was measured at 630 nm using a spectrophotometer. The concentration of NH_4^+-N was determined by reference to a standard curve (0-50 µg NH_4^+-N ml^{-1}) prepared from a standard solution of ammonium sulphate $(NH_4)_2SO_4$.

(a) <u>Standard ammonium solution:</u> was prepared by dissolving 0.4717 g ammonium sulphate $(NH_4)_2SO_4$ in 1 litre distilled water for (100 µg NH_4^+-N ml^{-1}).

(b) <u>Phenol solution:</u> was prepared by dissolving phenol (62.5 g) in ethanol (25 ml) and adding acetone (18.5 ml) to give a total of 100 ml. The phenol solution was stored in the dark at 4^{0}C.

(c) *<u>Phenolate reagent:</u> was prepared by mixing 20 ml of phenol solution with 20 ml caustic solution (27% NaOH w/v) and diluting to 100 ml. The reagent was prepared fresh daily.

(B) Analysis of nitrite-N (NO_2^--N) (Hesse, 1971)

Filtrate (2 ml) was added to a 50 ml volumetric flask, diluted with distilled water (40 ml) and *diazotising reagent (1ml) was added and incubated at room temperature for 5 minutes. **Coupling reagent (1ml) was added and the volume was made up to mark with distilled water. After 20 minutes incubation at room temperature, the intensity of the pink colour formed was measured at 520 nm using a spectrophotometer and the amount of nitrite was determined by reference to a calibration curve (0-10 μg NO_2^--N ml^{-1}) prepared from a standard solution of $NaNO_2$.

(a) Standard nitrite solution: was prepared by dissolving 0.4929 g sodium nitrite $NaNO_2$ (100 μg NO_2^- -N ml^{-1}) in 1 litre distilled water volumetrically.

(b) * Diazotising reagent: 0.5g of sulphanilamide was added to 2.5 N HCl (100 ml) and dissolved. The reagent was stored in an amber bottle in a refrigerator at 4^0C.

(c) Coupling reagent:** 0.3 g of N-(1-naphthyl)-ethylenediamine hydrochloride was dissolved in 0.1 N HCl (100 ml). The reagent was stored in an amber bottle in a refrigerator at 4^0C.

(C) Chromotropic acid method for nitrate-N determination (Sims and Jackson, 1971)

To filtrate (3 ml), *chromotropic acid reagent (7 ml) was added, mixed, cooled in cold water and incubated at 40^0C for 45 minutes. The intensity of the yellow CTA-NO_3 complex

was measured at 430 nm using a spectrophotometer. The NO_3^- - N concentration was determined by reference to a standard curve (0-5 µg NO_3^- -N ml^{-1}), prepared from a standard solution of KNO_3.

(a) <u>Standard Nitrate Solution:</u> 0.722 g Potassium nitrate (KNO_3) were dissolved in distilled water and made up to 1 litre volumetrically, for 100 µg NO_3^- -N ml^{-1}.

(b) <u>Chromotropic acid reagent</u> ($C_{10}H_6O_8S_2Na_2$):

A 0.1% (v/v) stock solution of chromotropic acid in concentrated sulphuric acid (H_2SO_4) was prepared by dissolving 1.84g chromotropic acid in litre H_2SO_4. This solution was stored in an amber bottle in a refrigerator at 4^0C for several months.

(c) <u>*A working chromotropic acid solution (CTA):</u>

A working CTA-solution (0.01% v/v) was prepared by diluting 100 ml of stock solution to 990 ml with concentrated sulphuric acid (H_2SO_4) then added 10 ml concentrated HCl using fume cupboard. This solution was stored at 4^0C for several weeks only.

3.2.4 Determination of dry weight

Soil (in triplicate) was dried at 60^0C overnight and the dry weight of the soil was determined.

3.2.5 Determination of pH

The pH of the soil solutions were determined by glass electrode pH meter.

3.3 RESULTS AND DISCUSSION

3.3.1 Effect of silicon compounds on nitrification in the agriculture loam soil

The effect of sodium silicate , silicic acid and rock potash on nitrification in the agricultural loam soil is shown in Fig 3.2 (a-c). Sodium silicate was the only silicon compound to have an pronounced, consistent effect on soil nitrate concentrations over the 28 day incubation period; the effect being to increase nitrate concentrations, by a factor of around 2.0-2.5. None of the added silicon compounds had a pronounced effect on soil nitrite concentrations (Fig. 3.2 b), but in addition to increasing nitrate concentrations, sodium silicate also increased ammonium concentrations at day 14 (Fig. 3.2 a).

As was seen in the Chapter 2, the addition of sodium silicate generally leads to an increase in soil pH, a finding confirmed by the results shown in Fig. 3.3. Since nitrification in agricultural loam soils generally increases with increase soil pH (Wainwright, 1974), the increases in nitrate concentrations seen following the addition of sodium silicate can be explained on the basis of a pH effect, rather than to a direct stimulatory effect of silicon on the process.

The above conclusion is given further credence by the data shown in Figs. 3.4 and 3.5. The data given in Figure 3.4 show that soil nitrate concentrations increase with increasing amounts of added sodium silicate, but not silicic acid and rock potash. Soil ammonium concentrations again increase with increasing amounts of added sodium silicate, but not following addition of the other two silicon compounds. An additional finding is that nitrite concentrations increase to, what are unusually high levels, for this ion in soil. Fig 3.5 again shows that the effects of sodium silicate on the concentrations of N-ions results from a resultant increase in soil pH.

It used to be assumed that soil nitrification was always a beneficial soil process. However, nitrification is now commonly regarded as being detrimental to both the environment and the N-economy of agricultural soils. This is because it leads to an increase in nitrate which can (a) be readily leached from the soil, (b) be lost via denitrification, (c) cause eutrophication in water courses and (d) cause pollution of water, leading to blue baby disease and possibly gastric cancers. As a result of these detrimental effects, nitrification is now often regarded as a somewhat detrimental process. Indeed, particularly in the USA, nitrification inhibitors (e.g. N-serve) are added to certain agricultural soils to inhibit this process. Rock potash and silicic acid did not stimulate nitrification in the agricultural loam studied here, and so could be added without any detrimental effect on soil fertility or the environment (at least in relation to this part of the N-cycle). Addition of sodium silicate, in contrast, increased the concentration of soil nitrate, ammonium and nitrite. The fact that nitrate formation is regarded as detrimental to soil fertility has already been commented upon. Although ammonium can be used as a N source by many plants, when produced in high concentrations, particularly at high soil pH (as seen following sodium silicate addition) this ion (NH_4^+) can be lost to the atmosphere by the process of ammonium volatilisation, thereby constituting a third

(the other two being nitrate leaching and denitrification) means by which sodium silicate addition could cause N to be lost from soils. The formation of the high concentrations of nitrite seen following sodium silicate amendment (especially at high application rates) could also be regarded as detrimental to soil fertility, since nitrite, even at lower concentrations than produced here, can be toxic to plants. As a result of these effects on nitrification, while the addition of rock potash and silicic acid to soils would appear not to be detrimental, the addition of sodium silicate to agricultural loam soils would appear to be

potentially detrimental, because of its ability to increase concentrations of soil ammonium, nitrate and nitrite.

FIG: 3.2

Effect of added silicon on nitrification (adding 100 µg NH_4^+-N ml^{-1}) in agricultural loam soil. Means of triplicates, ± Standard error.

(a) µg NH_4^+ - N g^{-1} dry weight soil.

(b) µg NO_2^- - N g^{-1} dry weight soil.

(c) µg NO_3^- - N g^{-1} dry weight soil.

—□— control (soil lacking added silicon)

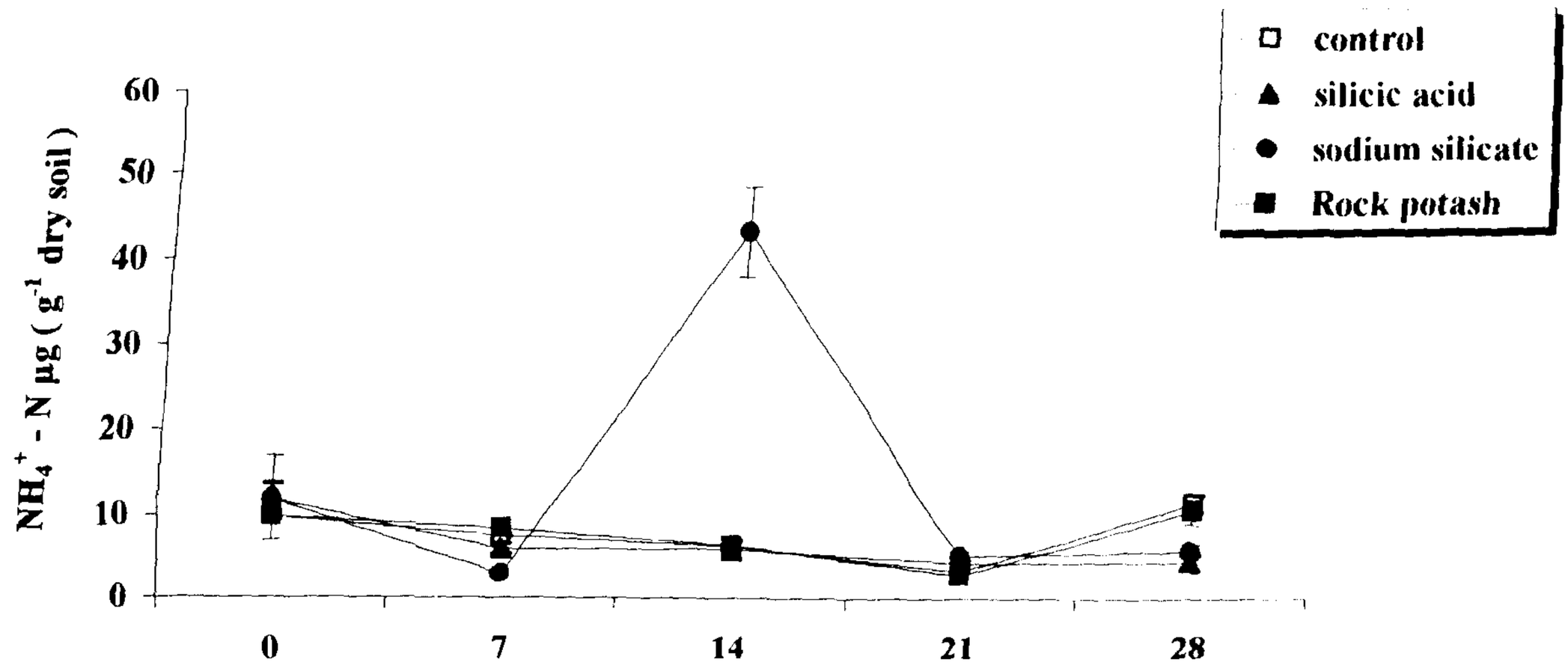
—▲— soil containing silicic acid

—●— soil containing sodium silicate

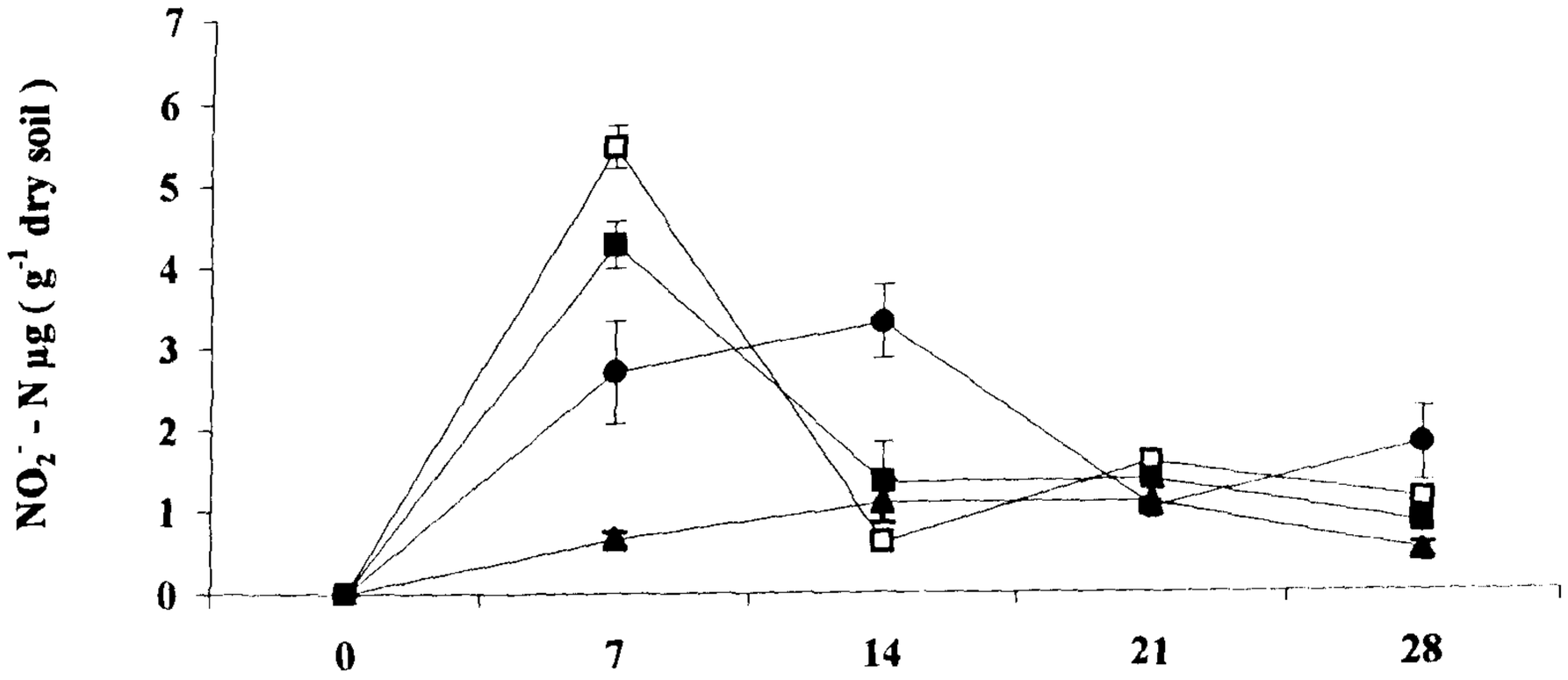
—■— soil containing rock potash

FIG: 3.2

(a)


control
silicic acid
sodium silicate
Rock potash
NH_4^+ - N µg (g^{-1} dry soil)
60
50
40
30
20
10
0
0
7
14
21
28

(b)


NO_2^- - N µg (g^{-1} dry soil)
7
6
5
4
3
2
1
0
0
7
14
21
28

(c)

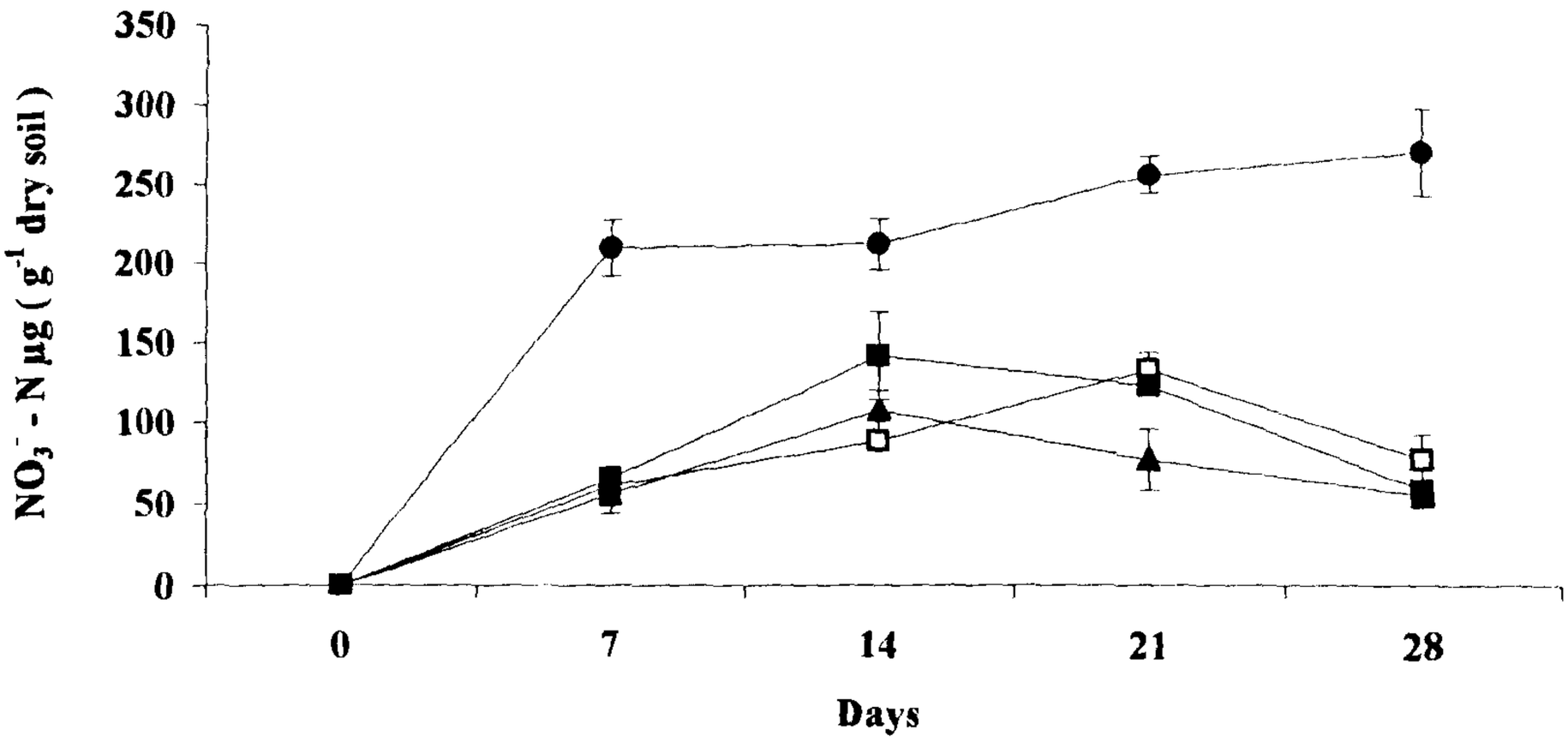

NO_3^- - N µg (g^{-1} dry soil)
350
300
250
200
150
100
50
0
0
7
14
21
28
Days

FIG: 3.3

Effect of added silicic acid, sodium silicate, and rock potash on the pH of agricultural soil, used for the determination of nitrification.

—□— control (soil lacking added silicon)

—▲— soil solution containing silicic acid

—●— soil solution containing sodium silicate

—■— soil solution containing rock potash

FIG: 3.3

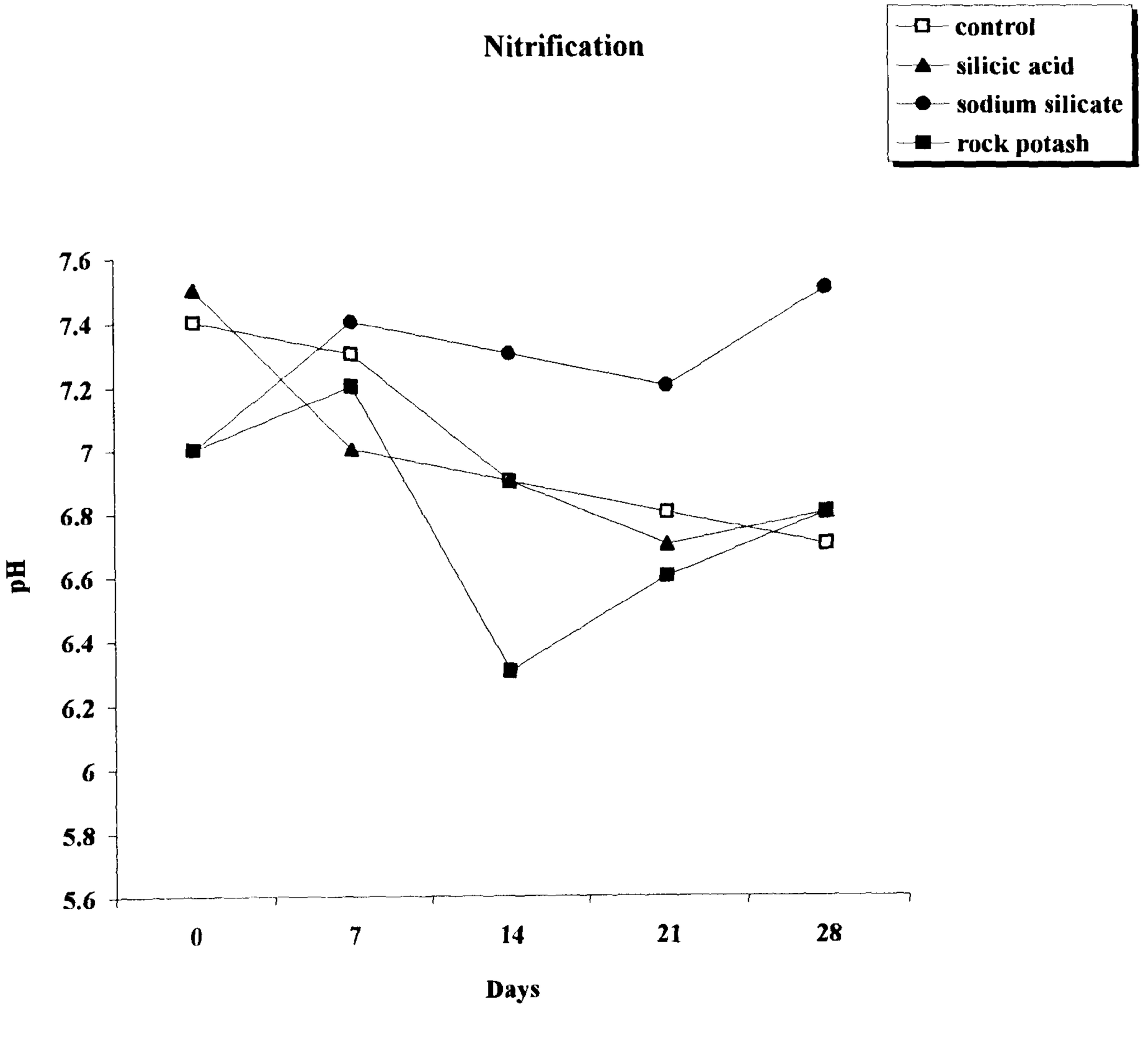

Nitrification
control
silicic acid
sodium silicate
rock potash
pH
7.6
7.4
7.2
7
6.8
6.6
6.4
6.2
6
5.8
5.6
0
7
14
21
28
Days

FIG: 3.4

Effect of 1, 5, 10 and 20 g of silicon compounds on nitrification (adding 100 µg NH_4^+-N ml^{-1}) in agricultural loam soil. (Means of triplicates, ± Standard error).

(a) µg NH_4^+ - N g^{-1} dry soil

(b) µg NO_2^- - N g^{-1} dry soil

(c) µg NO_3^- - N g^{-1} dry soil

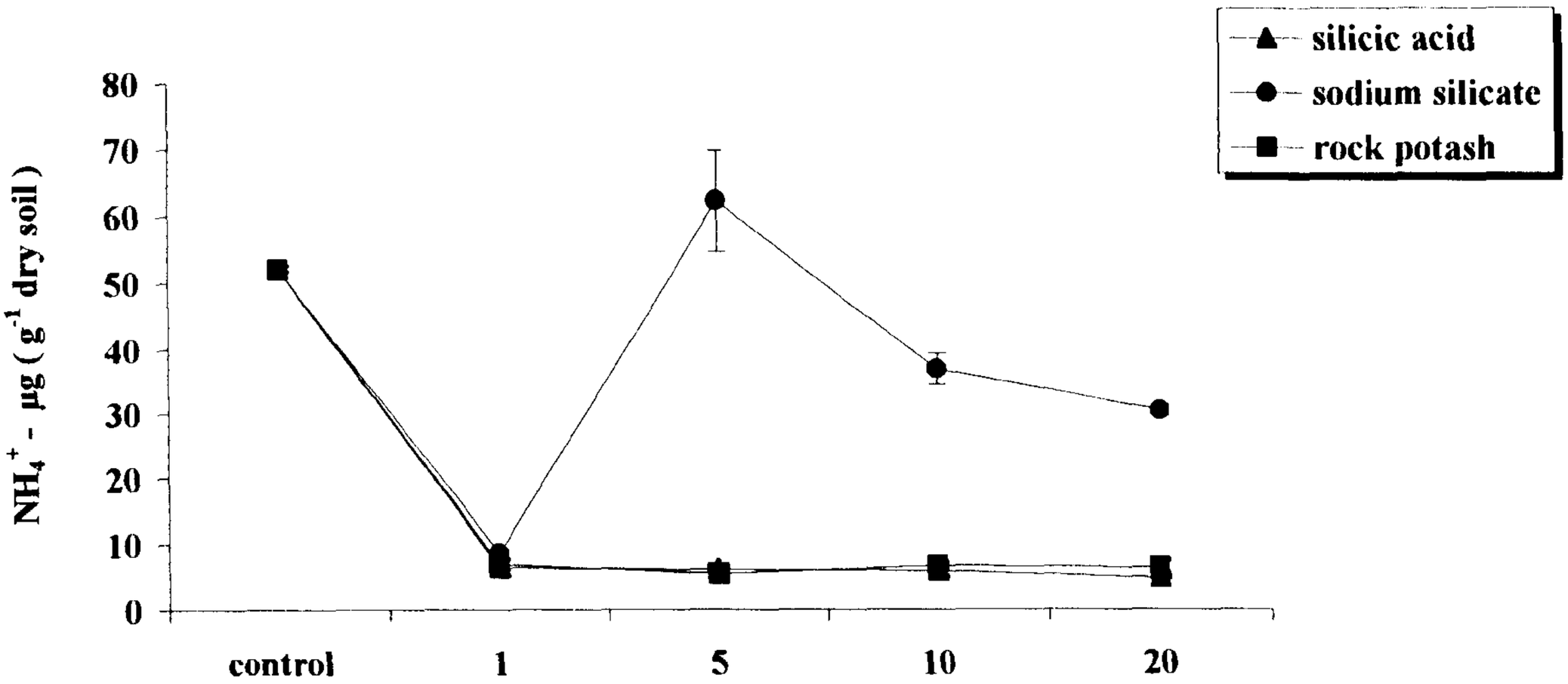
——▲—— soil containing added silicic acid

——●—— soil containing added sodium silicate

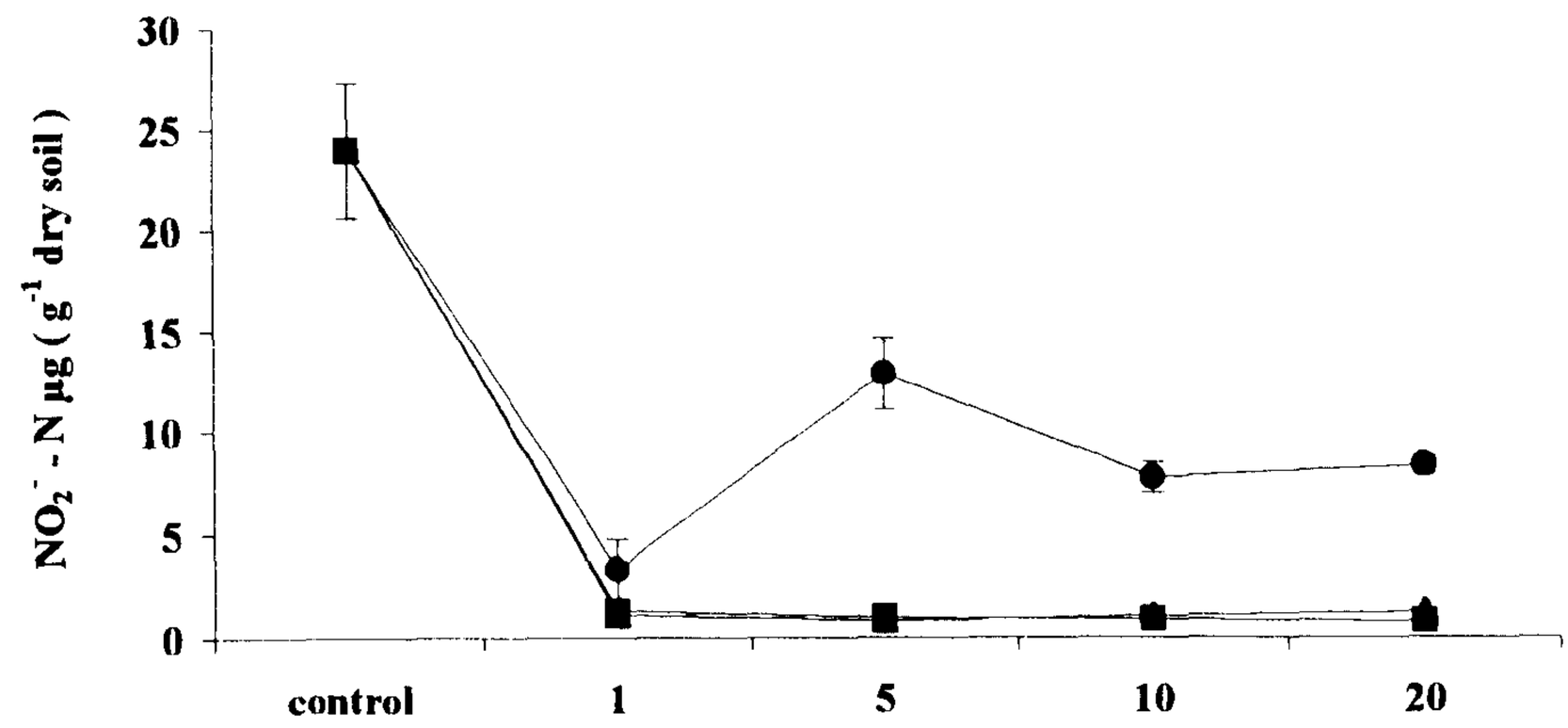
——■—— soil containing added rock potash

FIG: 3.4

(a)


silicic acid
sodium silicate
rock potash
NH_4^+ - µg (g^{-1} dry soil)
80
70
60
50
40
30
20
10
0
control
1
5
10
20

(b)


NO_2^- - N µg (g^{-1} dry soil)
30
25
20
15
10
5
0
control
1
5
10
20

(c)

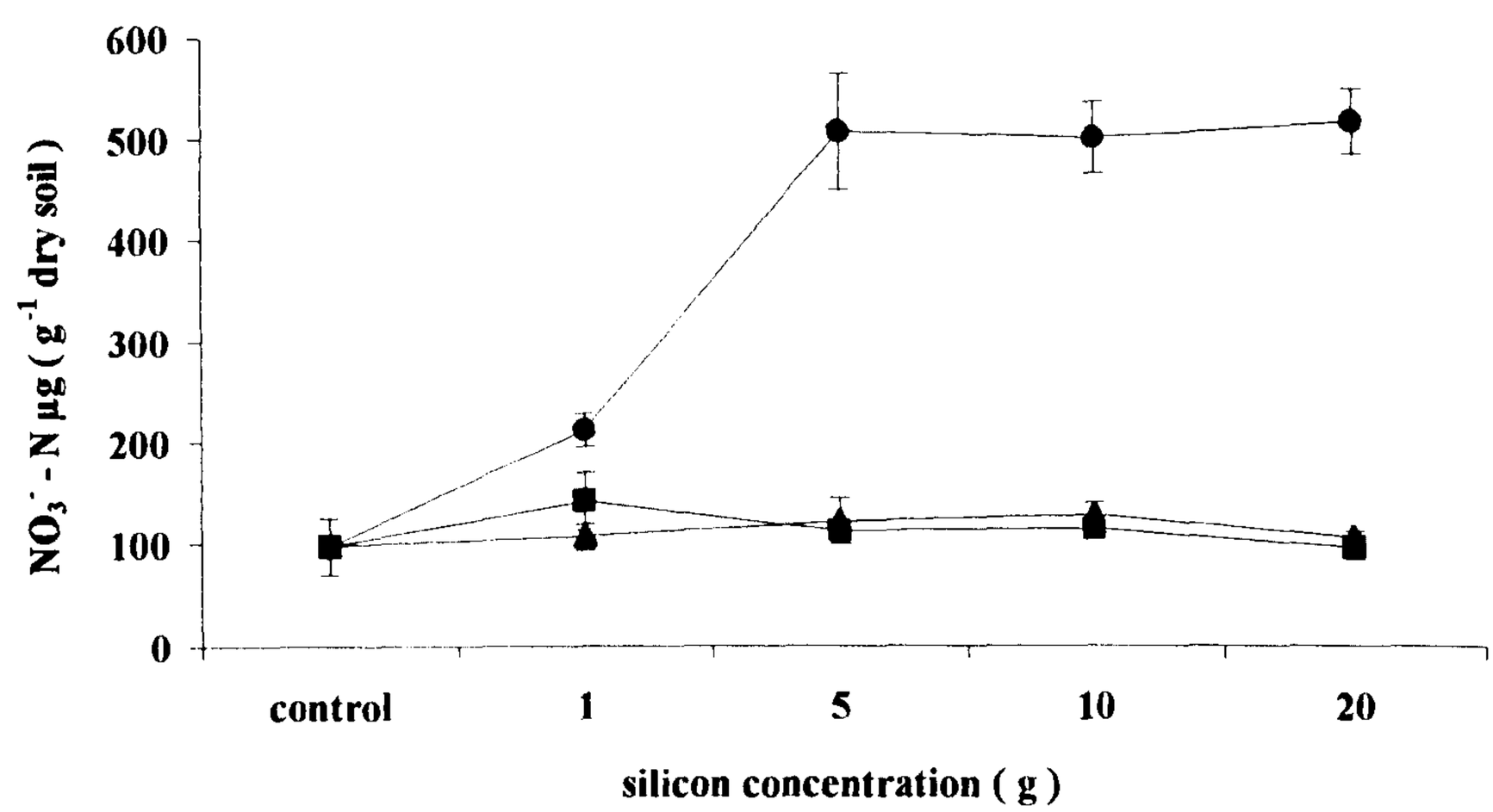

NO_3^- - N µg (g^{-1} dry soil)
600
500
400
300
200
100
0
control
1
5
10
20
silicon concentration (g)

FIG: 3.5

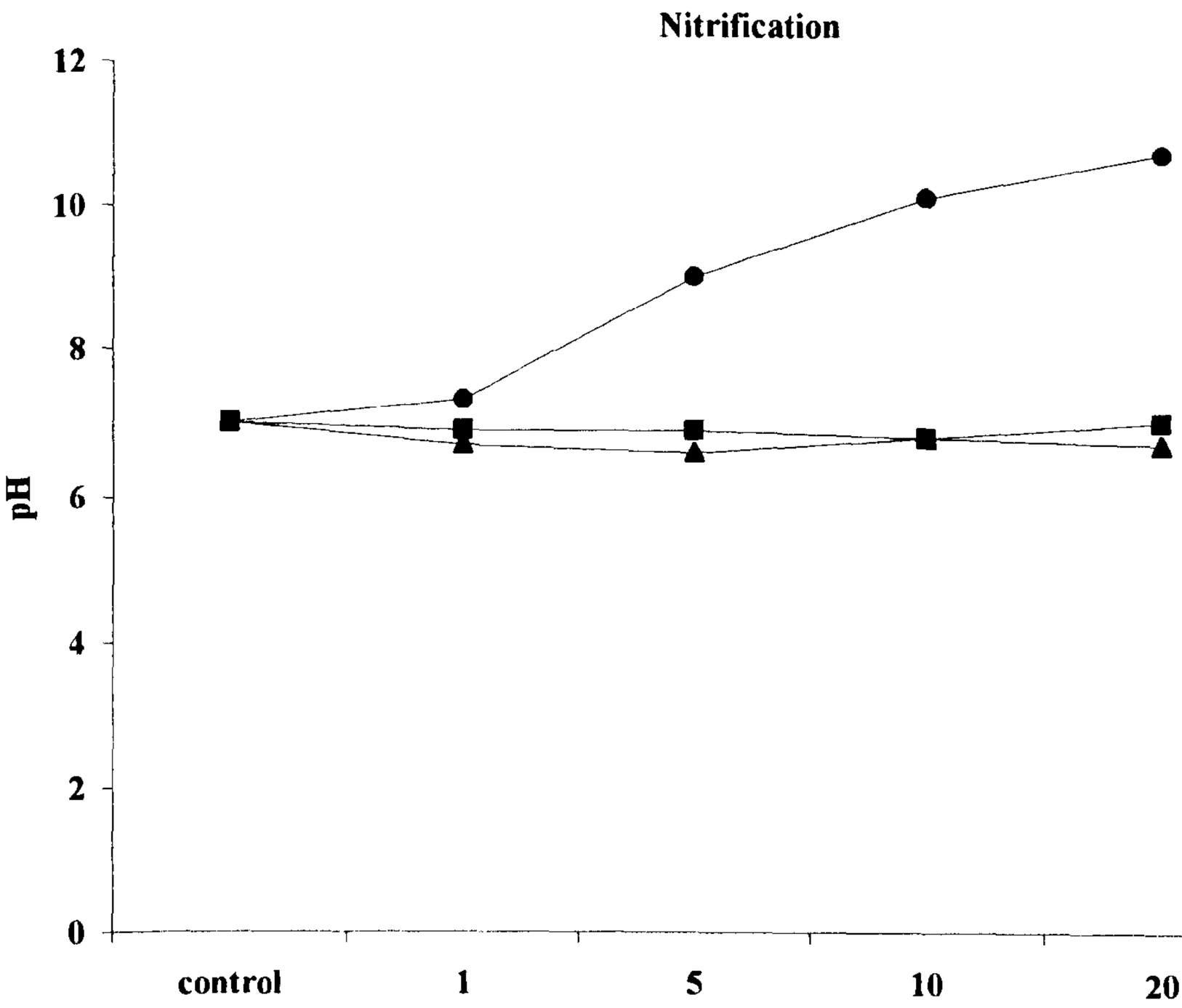
Effect of different amounts of silicic acid, sodium silicate, and rock potash on the pH of agricultural soil, used for the determination of nitrification.

——▲—— soil containing added silicic acid

——●—— soil containing added sodium silicate

——■—— soil containing added rock potash

FIG: 3.5


Nitrification
12
10
8
6
4
2
0
pH
control
1
5
10
20
silicon concentration (g)

CHAPTER FOUR

EFFECT OF SILICON ON SULPHUR OXIDATION IN SOIL

CHAPTER 4- EFFECT OF SILICON ON SULPHUR OXIDATION IN SOIL

****

4.1 INTRODUCTION

4.1.1 Sulphur cycle

Sulphur (S) is an essential nutrient required by plants, animals, humans and microorganisms. This element is widely distributed in nature. It is found in the sun, stars, meteorites, the ocean, the earth, and in all life forms. It is estimated that sulphur is the ninth most abundant element in the universe. Despite its availability in large amounts in the earth's crust, sulphur supply in the soil is insufficient for the growth of plants. Sulphur deficiencies are found to occur in soils throughout the world (Bixby and Beaton, 1970). Sulphur may be obtained from the weathering of soil minerals, from the atmosphere, and from organically bound sulphur. on the earth's surface it is available as elemental sulphur, sulphides (reduced), and sulphates (oxidised). The most important sulphides are iron pyrite, FeS_2; chalcopyrite, $CuFeS_2$; sphalerite, ZnS; and galena, PbS and the naturally occurring sulphates are anhydrite, $CaSO_4$; gypsum, $CaSO_4.2H_2O$ and kieserite, $MgSO_4.H_2O$. Plants contain as much sulphur as phosphorus, and sulphur is as important as nitrogen in the formation of proteins (Killham, 1994). Sulphur and nitrogen, both are constituents of protoplasm. Plants obtain their sulphur containing amino acids (e.g. cystine and methionine) by reducing dissolved sulphate. Sulphur is also an important component of several organic compounds that form enzyme systems and B vitamins, thiamine, biotin, and lipoic acid. It is also

available in the tissues and excretory products of animals as free sulphates, taurine, and rarely thiosulphate and thiocyanate. Sulphur occurs in the soil in both organic and inorganic states and is readily metabolised in soil. The inorganic fraction is comparatively smaller than the organic. It is assumed that over 90% of the sulphur in most non-calcareous, non-tropical, surface soils is in organic forms, about half in the form of sulphate esters and esters with C–O–S linkage (Tisdale *et al.*, 1985), about 20% in the form of sulphur directly bonded to carbon such as sulphur containing amino acids (Biederbeck, 1978). Sulphur ranges from 20-2000 $\mu g\ g^{-1}$ in most of the agricultural soils and organic, volcanic ash and tidal-marsh soils contain more than 3000 $\mu g\ g^{-1}$ and some, the desert soils contain 10,000 $\mu g\ g^{-1}$ (Paul, 1996). Sulphur inputs in the soil can occur through the deposition in rain and snow, in dry particulates and by direct absorption of the gases. Sulphur input varies from up to 100 kg S $ha^{-1}\ y^{-1}$ in areas close to industrial pollution sources to less than 5 kg S $ha^{-1}\ y^{-1}$ in rural areas distant from these sources (UNEP, 1991). By far the dominant form of sulphur taken up by plants and soil microbes is sulphate (SO_4^{2-}). Sulphur like nitrogen, undergoes several transformations in soil which together form the sulphur cycle (Fig. 4.1). The reactions that occur in sulphur cycle, are largely mediated by micro-organisms (Brown, 1982). Sulphur transformations in the soil are briefly given as (a) sulphur oxidation (b) sulphur reduction, (c) protein synthesis (d) decomposition of protein and its sulphur containing derivatives.

SOIL SULPHUR CYCLE

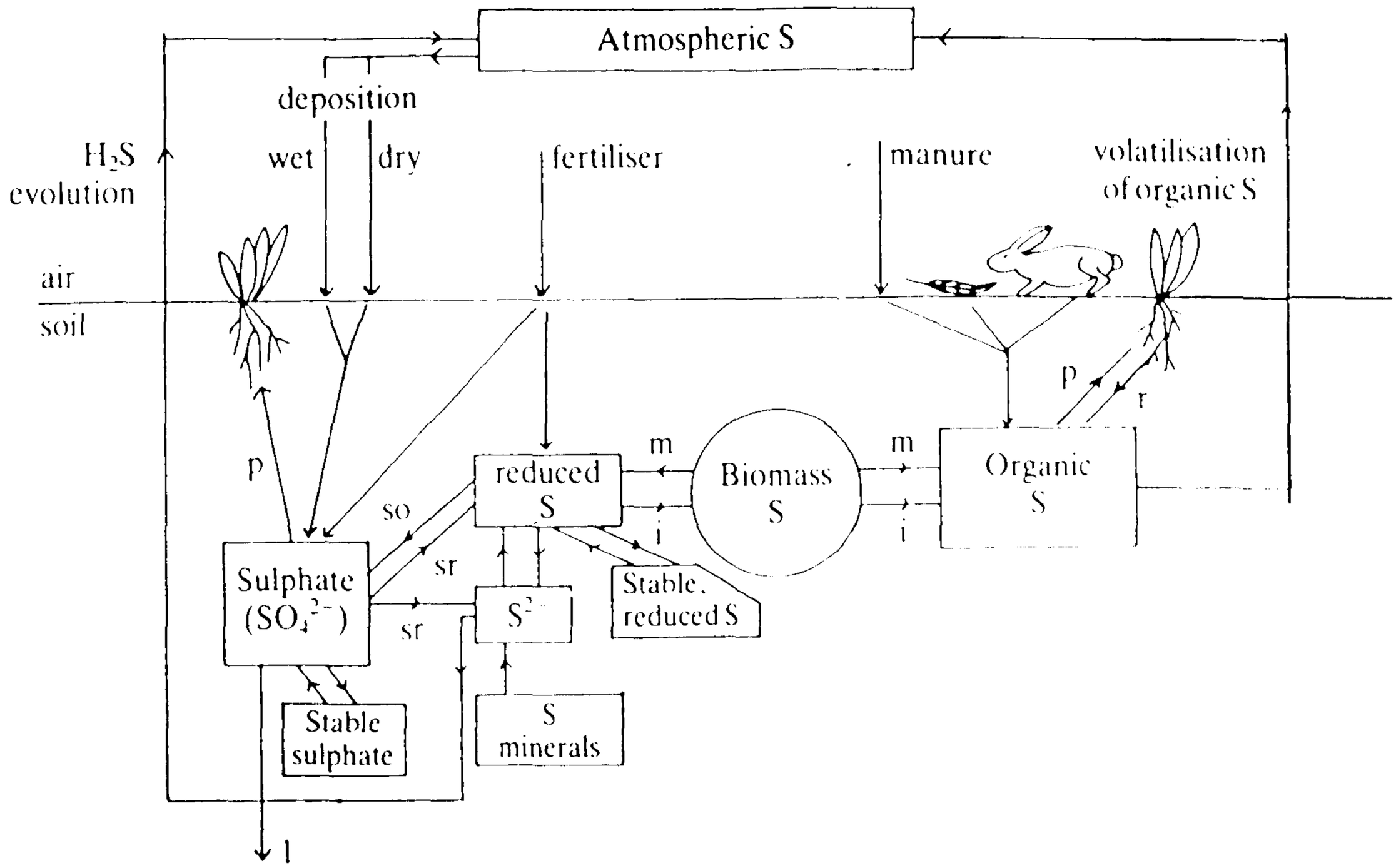


FIGURE 4.1. Abbreviations: i, immobilization; m, mineralization; p, plant uptake; r, root exudation and turnover; so, oxidation and subsequent leaching (l); sr, reduction. (From Killham, 1994)

4.1.2 Mineralisation

Sulphur mineralisation is the conversion of sulphur from organic to inorganic forms by biological and chemical processes. It also plays a major role in the metabolism of macro-organisms. Sulphur mineralisation is therefore an important means by which sulphate is mobilised in soils as well as acting as a source of H+ ions (Tabatabai, 1985). The mechanisms, responsible for sulphur mineralisation are not fully known but it has been suggested that two mechanisms (1) biological and (2) biochemical are involved in sulphur mineralisation (McGill and Cole, 1981).

(1) Biological mineralisation

Sulphur, is present in amino acids in the proteins of plants, animals, and microbes and act as substrate, that can be utilized by microorganisms. Carbon-bonded sulphur (e.g. amino acids) is mineralised by micro-organisms during the oxidation of carbon for energy requirement (Killham, 1994). Some of the inorganic products are utilized by soil micro-organisms for cell synthesis and the remainder is released into the environment. Under aerobic conditions, the end product of inorganic sulphur oxidation is sulphate whereas, decomposition of proteinaceous matter, under anaerobic conditions results in the formation of hydrogen sulphide (H_2S) and odoriferous mercaptans in soil (Alexander, 1977).

(2) Biochemical mineralisation

This process occurs when non-carbon-bonded organic sulphur (ester sulphates) is mineralised through the enzymatic catalysis (sulphatases, formed by micro-organisms) external to the cell membrane (Killham, 1994). Mineralisation increases in the presence of oxygen, temperature in the mesophilic range, (Tabatabai and Al-Khafaji, 1980); moisture level and the addition of lime to acid soils (Williams, 1967).

4.1.3 Sulphur oxidation

The oxidation of reduced sulphur in soil is usually regarded as a microbial process (Wainwright, 1978a), although some non biological oxidation of the element has been demonstrated in sterile soils (Nor and Tabatabai, 1977; Wainwright and Killham, 1980). In the presence of available electron acceptors, sulfide, elemental sulphur, thiosulphate, tetrathionate, trithionate and sulphite are oxidised by both chemical and biological pathways:

$$SH^- \rightarrow S_0 \rightarrow S_2O_3^{2-} \rightarrow S_4O_6^{2-} \rightarrow S_3O_6^{2-} \rightarrow SO_3^{2-} \rightarrow SO_4^{2-}$$

Sulphide elemental thiosulphate tetrathionate trithionate sulphite sulphate

4.1.3.1 The role of microorganisms in sulphur oxidation

A variety of micro-organisms are capable of oxidising sulphur in the environment including members of the genus *Thiobacillus*, a number of heterotrophs, the photosynthetic sulphur bacteria, and the colourless, filamentous sulphur bacteria (Burke *et al.* 1974). Colourless S-oxidising bacteria and their characteristics are presented in the tables 4.1 and 4.2. Wainwright, (1978b) has commented, that in most soils only the thiobacilli, and heterotrophs play an important role in sulphur oxidation, the exceptions being flooded soils where the aquatic bacteria predominate. These microorganisms are divided into three groups, (1) The chemotrophic sulphur bacteria (2) heterotrophic sulphur bacteria and (3) phototrophic sulphur bacteria.

(1) <u>The chemotrophic sulphur bacteria</u>

This group of bacteria is variable in both morphology and physiology ranging from specialist obligate chemolithotrophs via facultative chemolithotrophs which can grow mixotrophically, to specialist heterotrophs, some of which may not benefit directly from the oxidation of reduced sulphur compounds (Kuenen and Beudeker, 1982). It is the most studied group of soil sulphur oxidisers. Out of the nine species of thiobacilli, five have been studied in detail. In most soils, oxidation is dominated by the thiobacilli. These bacteria are generally gram negative, nonsporulating rods, deriving their energy from the oxidation of sulfides, elemental sulphur, thiosulphate, tetrathionate and thiocyanate, while CO_2 or bicarbonate supplies the carbon for chemoautotrophic growth. (London and Rittenberg, 1967). In addition they can be subdivided into those growing on neutral pH and those

which live at acidic pH. They can also grow both at acidic and alkaline pH values but Vitolins and Swaby (1969) reported that thiobacilli are important sulphur oxidisers only at pH values below 7, while heterotrophs are the primary sulphur oxidisers in neutral to alkaline soils (pH 6.0-7.5). The reduction in pH resulting from sulphuric acid formation by thiobacilli may also control some diseases of plants such as potato scab (Brown, 1982). Thiobacilli have been found in all soils in great numbers, particularly in soils receiving applications of sulphur as a fertilizer, either in organic (sewage, etc.) or inorganic forms. Finely-powdered elemental sulphur is a useful source of fertilizer sulphur, being readily oxidisable in soil to plant-available sulphate yet possessing some slow release characteristics (Chapman, 1997). Thiobacilli are generally regarded as the major sulphur oxidisers in agricultural soils. Incorporation of elemental sulphur or reduced forms of sulphur compounds in soil, increases dramatically the numbers of thiobacilli. (Burns, 1967; Alexander, 1977; Adamczyk-Winiarka *et al.*, 1975). Wainwright, (1984a) mentions that there are reports of soils which are deficient in thiobacilli but which are still able to oxidise sulphur. LopezAguirre, (1999) demonstrates that sulphur application and/or leaching had an increasing effect on the populations of putative nitrogen-fixers, *Thiobacillus thioparus*-like, *T. thiooxidans*-like and total bacterial population also increased when sulphur was added, however, populations of fungi and actinomycetes decreased in soils amended with sulphur while putative nitrogen-fixing organisms were unaffected. Despite these, however, there have been few studies on the species composition, distribution and autecology of sulphur oxidising micro-organisms (Wainwright, 1984a).

TABLE (4.1) **COLOURLESS SULPHUR OXIDISERS.**

(Modified after Kuenen and Beudeker, 1982)

Obligate chemolithotrophic S-bacteria	**Facultative chemolithotrophic S-bacteria**
(a) Aerobic bacteria	
Thiobacillus thiooxidans	*T. novellus*
T. neapolitanus	*T. intermedius*
T. ferrooxidans	*T. acidophilus*
T. kabobis	*T. organoparus*
T. tepidarius *	*Sulfolobus acidocaldarius*
Thiomicrospira pelophila	*Sulfolobus brierleyi*
(b) Facultative anaerobic bacteria	
T. denitrificans	*Thiobacillus A2*
T. thioparus	*Thermothrix thiopara*
Thiomicrospira denitrificans	*Paracoccus denitrificans*
	Thiosphaera pantotropha **

Chemolithoheterotrophs	**Heterotrophs**	**Unclassified**
T. perometabolis	*Beggiatoa spp.*	*Thiovulum*
Pseudomonas sp.	*Pseudomonas spp.*	*Thiophysa*
		Thiothrix
		Thiospira
		Thioploca

(* From: Wood and Kelly, 1985; ** From: Robertson and Kuenen, 1983)

TABLE (4.2) **CHARACTERISTCS OF SOME CHEMOTROPHIC SULPHUR - BACTERIA. (Modified from Paul, 1996)**

Bacterial characteristics

Eubacteria

Family *Thiobacillaceae:* All gain energy from S oxidation, and reduce CO_2 by the Calvin cycle.

Thiobacillus: Motile, nonspore forming rods (0.3x1-to3- µm)
Thiomicrospira. Motile spirilloid cells
Thiosphaera. Nonmotile spherical cells sometimes in chains.

Thiobacillus thiooxidans. Strictly aerobic, motile, gram negative, has a pH growth range 2 to 5.
Thiobacillus thioparus. Grows best at 25-30^{0}C; optimum pH 5 to 8. Some strains grow on thiocyanate.
Thiobacillus denitrificans. Similar to T. thioparus, can substitute NO_3- for O_2 as an electron acceptor with the loss only to the atmosphere.
Thiobacillus ferrooxidans. A strict aerobic bacterium, pH ranging from 1.5 to 5, oxidizes ferrous iron (Fe2+) as a source of energy and is of major significance in the production of acid mine water and the commercial leaching of ores.
Thiobacillus intermedius. A facultative chemolithotroph, pH 3 to 7, capable of using $S_2O_3^{2-}$ as an electron donor. Growth is stimulated by the presence of organic matter.

Family *Beggiatoaceae*: Multicellular, gram negative filamentous, gliding motility and S inclusions.
Beggiatoa. Single filaments.
Thioploca. Filaments in bundles enclosed in sheath.
Thiothrix. Attached rigid filaments, produce gonidia.

Archaebacteria

Family *Sulfolobaceae:* Extremely thermoacidophilic with no peptidoglycan in cell wall, contain isoprenyl ether membrane lipids.
Sulfolobus. Irregular nonmotile coccoids.
Acidianus. Irregular nonmotile coccoids which also reduce S to HS^-

(2) Heterotrophic sulphur-oxidizers

Sulphur oxidation by heterotrophic bacteria was demonstrated by Guittoneau (1927), and later, Starkey (1934) confirmed that heterotrophic bacteria isolated from soil could oxidise thiosulphate to sulphate via tetrathionate. The involvement of heterotrophic microorganisms in sulphur-oxidation in soil, however, has become increasingly recognised, (Killham, 1994). A wide range of hetrotrophic bacteria (Friedrich and Mitrenga, 1981), fungi (Killham *et al.*, 1981) and actinomycetes (Yagi *et al.*, 1971) can oxidise various forms of reduced sulphur *in vitro.* The bacterial species of the genera *Arthrobacter, Achromobacter, Bacillus, Beggiatoa Flavobacterium, Micrococcus, Mycobacterium, Pseudomonas, Sphaerotilus* are capable of oxidizing the sulphur. Unlike the S-oxidising autotrophs, heterotrophic bacteria do not appear to gain energy from the oxidation of sulphur (Trudinger, 1967; Schook and Berk, 1978). Various common heterotrophic soil bacteria (*Bacillus mycoides, B. fluorescens*) are capable of oxidising small amounts of elemental sulphur, in nutrient solutions containing organic nitrogen and sources of energy (Alexander, 1977). Heterotrophs are believed to be the primary S-oxidisers in neutral and alkaline soils, (Paul, 1996). Heterotrophic S-oxidation seems to be exactly the same as heterotrophic nitrification for the following reasons, as mentioned by Killham (1994),

1- Neither process has been shown to be energy linked.

2- S-oxidising and N-oxidising heterotrophs can utilise both inorganic and organic forms of sulphur and nitrogen, unlike their autotrophic counterparts.

3- Although their exact role and significance in soil is uncertain, it seems that heterotrophic S-oxidisers and nitrifiers only dominate certain specific soil environments that favour their growth/activity at the expense of the autotrophs.

The ability of fungi to oxidise S has long been studied by Armstrong (1921); and Abbot, (1923), but has only recently been emphasized (Wainwright and Killham, 1980; Germida, 1985; Grayston *et al.*, 1986). Some heterotrophic S-oxidising fungi are given in the Table 4.3. Fungi such as *Aspergillus niger, Penicillium cyclopium* and *Botrytis cinerea* which were shown to oxidise thiosulphate to sulphate, *in vitro*, occasionally forming tetrathionate as intermediate. Wainwright, (1984b, 1984a) reported that the soil fungi have mainly appeared to be capable of sulphur oxidation, but thermophilic marine fungi like *Asteriomyces cruciatus* are also involved. Killham *et al.* (1981) reported that sulphur oxidation carried out by *Aureobasidium pullulans* is an enzymatic process, and it is therefore unlikely that the process is fortuitous and incidental to normal metabolism. However fungi are regarded as strict heterotrophs and unlikely to gain energy from sulphur oxidation. Armstrong (1921) also reported biomass increases when fungi are grown in thiosulphate. The growth of hyphae of vesicular arbuscular mycorrhizal fungus *Glomus calledonium* is also stimulated by thiosulphate, metabisulphate, and sulphate (Hepper, 1984).

Filamentous fungi and yeasts oxidise powdered sulphur, and several heterotrophic bacteria convert thiosulphate to tetrathionate in the presence of organic nutrients (Alexander, 1977). Wainwright (1984a) summarized the advantages which could be gained by fungi from sulphur oxidation.

a) Fungi oxidise sulphur to sulphate to meet their nutritional requirements for S. However large quantities of free sulphates are formed as the result of chemical and microbial oxidation

of sulphur, which is present in the environment. In this case fungi have no need to oxidise sulphur for nutrition.

b) The formation of thiosulphate and tetrathionate during sulphur oxidation, can protect fungi from the toxic effects of heavy metals when growing *in vitro* (Wainwright and Grayston, 1983). These ions function as ligands, complexing metals or reducing to thiols and making them unavailable.

c) Many fungi accumulate elemental sulphur which acts as a self-inhibitor of spore germination (Pezet and Pont, 1977). Fungal sulphur-oxidation would remove such germinaton inhibitors.

d) Fungi may avoid sulphur toxicity by oxidising the elemental sulphur to sulphate (Tweedy, 1969). Fungi can reduce the elemental sulphur to H_2S, which is toxic in nature, therefore these powerful toxins can be removed by the oxidation of elemental sulphur and H_2S. Skerman *et al.* (1957) suggested a similar mechanism for toxic-protection to explain the ability of the heterotrophic bacteria *Sphaerotilus natans* and *Beggiatoa* sp. to oxidise H_2S. Pepper and Miller (1978) reported that the rate and magnitude of heterotrophic oxidation was similar to that of *Thiobacillus thiooxidans* under optimum conditions.

TABLE (4.3) HETEROTROPHIC SULPHUR OXIDISING FUNGI.

Fungi	References
Aspergillus niger *Penicillium cyclopium* *P. glaucum* *Botrytis cinerea*	Armstrong (1921)
Fusarium solani	Wainwright and Killham (1980)
Aureobasidium pullulans	Killham *et al.* (1981)
Alternaria tenuis *Cephalosporium sp.* *Penicillium decumbens* *Sporotrichium thermophile* *Asteriomyces crucicatus*	Wainwright (1984a)
Geosmithia argillacea *G. emersonii* *Myceliophthora thermophila* *Absidia glauca* *Aspergillus fumigatus* *Fusarium episphaeria*	Wainwright (1984b)
F. tricinctum *Mortierella isabellina* *Penicillium pinetorum* *Trichoderma hamantum* *T. viride* *Zygorhynchus moelleri* *Z. vuilmanii*	Germida (1985)
Trichoderma harzianum *Mucor flavus*	Grayston *et al.* (1986)

(3) The phototrophic sulphur bacteria

The phototrophic sulphur bacteria have been found under anaerobic environments, e.g. in H_2S containing mud and stagnant waters, which remain exposed to light. They are also present under extreme conditions of salinity and high temperatures living in sulphur springs and saline lakes as a coloured layer under salt deposits (Paul, 1996). They occur in larger numbers in shallow waters than in soils. However Wainwright (1984a), mentioned that the phototrophic bacteria have a major role in the oxidation of reduced sulphur in rice paddy soils. These bacteria are classified into two groups on the basis of their pigmentation and photosynthetic pathways. (a) The green sulphur bacteria (Chlorobiaceae) and (b) the purple sulphur bacteria (Chromatiaceae). Both groups, include cocci, vibrios, rods, spirals, budding and gliding gram negative bacteria. The green sulphur bacteria possess green colour due to bacteriochlorophyll or brown colour due to presence of carotenoids. The members of the family Chlorobiaceae, oxidise H_2S to sulphate, forming extra cellular sulphur granules in the presence of high H_2S concentrations. The purple sulphur bacteria vary in colour ranging from bluish violet, purple, deep red, and orange due to carotenoid pigments which dominate the bacteriochlorophylls in colour. The bacterial genera (Particularly *Chromatium, Thiosprillum, and Thiocapsa*), of the family Chromatiaceae, oxidise H_2S, thereby store sulphur globules intracellularly, which can be oxidised to sulphate (Zinder and Brock, 1978; Paul, 1996).

4.1.3.2 Environmental influence on S-oxidation

A number of environmental factors are responsible for affecting sulphur-oxidation in soils such as temperature, pH, moisture, by fertilizer, and soil-microbial factors e.g. populations of S-oxidisers and the effect of sulphur additions on the population (Janzen *et al.*, 1982).

(1) Temperature: It often affects the rate of sulphur oxidation, the process which occurs best at optimum temperatures ranging between 25 – 40^{0}C (Burns, 1967; Nor and Tabatabai, 1977; Skiba and Wainwright, 1984a). However some thermophilic bacteria (e.g. *Sulfolobus acidocaldarius*) and fungi can grow at temperatures above 40^{0}C. (Marsh and Norris, 1983; Wood and Kelly, 1985; Fliermans and Brock, 1972; Wainwright, 1984b).

(2) PH: Sulphur oxidation takes place at the range of pH 2 – 9 but can be enhanced with rising pH and consequently it tends to be stimulated by liming (Adamczyk-Winiarka *et al.*, 1975; Lettl *et al.*, 1981b). Vitolins and Swaby (1969), suggested that pH can control the distribution of S-oxidisers, with thiobacilli activated below pH 7 and heterotrophs by neutral to alkaline soils.

(3) Moisture: Plays an important role in S-oxidation. It has been shown that rates of sulphur oxidation are found to be maximum at soil-moisture content close to field capacity (Burns, 1967; Moser and Olson, 1953).

(4) Fertilizer: As studies on sulphur-fertilizer have revealed that the rate of sulphur-oxidation increases as the particle size is decreased (Wainwright, 1978a; Janzen *et al.*, 1982). The oxidation rate of fertilizer-S can also be affected by placement

(Janzen and Bettany, 1986) and increases with the rate of its application (Lettl *et al.*, 1981a).

(5) Organic matter: Stimulates, suppresses, or has no effect on S-oxidation (Swaby and Vitolins, 1969). Pepper and Miller (1978) mentioned that the addition of glucose to autoclaved soils containing thiosulphate, stimulate S-oxidation by heterotrophs but inhibit chemoautotrophic oxidation because of the formation of pyruvate.

(6) Soil-microbial influence: Sulphur oxidation being a primarily biological reaction in soil, is largely affected by the size and composition of the soil–microbial population.

4.1.4 Dissimilatory (respiratory) sulphate reduction

This process is largely carried out by some heterotrophs, which use sulphate as an electron acceptor in their anaerobic respiration. Sulphate reduction is increased by organic matter. Organic compounds such as choline, formate lactate, maleate, pyruvate and alcohols, serve as hydrogen donors in sulphate reduction (Killham, 1994). Obligate anaerobic bacteria of the genera *Desulfotomaculum, Desulfovibrio and Desulfobacter* achieve sulphur-reduction in the soil. Certain other micro-organisms are also capable of reducing sulphate to sulphide. Actinomycetes and fungi can further reduce partially reduced inorganic sulphur compounds (e.g. thiosulphate, tetrathionate and sulphite to sulphide) (Alexander, 1977). An interesting ecological niche for sulphate reduction is in the outer rhizosphere of paddy rice plants.

4.2 MATERIALS AND METHODS

4.2.1 Effect of different amounts of silicic acid and rock potash on sulphur-oxidation in agricultural soil in 14 days

Agriculture soil was collected (10 cm deep) from Sheffield.

To triplicate samples of soil (100 g, sieved <4mm), added, 1g elemental sulphur, 1, 5, 10 and 20g each of silicic acid and rock potash, plus 2 ml deionized water (sterile) mixed thoroughly in polythene bags. Control was set-up lacking added silicon compounds. All the soil samples were then incubated aerobically, at 25^0C for 14 days. Analysis was made by adding 1g soil to 20 ml deionized water (sterile) and shaken at 70 rpm for 15 minutes using orbital shaker. Soil solution was then filtered by Whatman No.1 filter paper. Clear filtrate was analysed for sulphate-S by using turbidimetric analysis.(section 4.2.4)

4.2.2 Effect of different amounts of silicic acid, sodium silicate and potassium silicate on sulphur-oxidation in agricultural soil in 14 days

To 100g triplicate set of fresh soil (Sieved <4mm), 1g elemental sulphur, 1, 5, 10 and 20g each of silicic acid, sodium silicate and potassium silicate, plus 2 ml deionized water (sterile) were added and mixed thoroughly in polythene bags. Control was set-up without silicon compounds added. All the soil samples were then incubated aerobically, at 25^0C for 14 days. Analysis was made by diluting 1g soil to 20 ml lithium chloride (0.1M) solution in screw capped glass bottles (150 ml) and shaken at 70 rpm by using orbital shaker for 15

minutes. The soil solution was then filtered by Whatman no:1 filter paper. Clear soil extract was analysed for sulphate-S by using turbidimetric analysis. (section 4.2.4)

4.2.3 Effect of different amounts of silicic acid, sodium silicate and potassium silicate on sulphur-oxidation in agricultural soil for four weeks

To 100g triplicate samples of agricultural soil (Sieved <4mm), 1g elemental sulphur, 1, 5, 10 and 20 g each of silicic acid, sodium silicate and potassium silicate, plus 2 ml deionized water (sterile) were added into polythene bags and mixed thoroughly. Control was set-up lacking added silicon compounds. All the soil samples were then incubated aerobically, at 25^0C for 7, 14, 21 and 28 days. Analysis was made by diluting 1g soil to 20 ml lithium chloride (0.5M) solution in screw capped glass bottles (150 ml) and shaken at 70 rpm by using orbital shaker for 15 minutes. The soil solution was then filtered by Whatman no:1 filter paper. Clear soil extracts were analysed for sulphate-S by using turbidimetric analysis. (section 4.2.4)

4.2.4 Analysis of inorganic sulphur ions

(a) <u>Turbidimetric analysis of sulphate-S (Hesse, 1971)</u>

To 5ml filtrate in 25ml volumetric flask, 1g $BaCl_2 . 2H_2O$ and 2ml of gum-acacia (0.25%w/v), were added and mixed, and the volume was made up-to 25 ml with distilled water. The white suspension resulting from precipitation of barium sulphate, was measured at 470 nm by spectrophotometer. The concentration of SO_4^{2-}- S was determined by reference to a standard curve (0 –100 µg SO_4^{2-} - S ml^{-1}) prepared from a standard solution of $Na_2SO_4.10H_2O$.

(b) <u>Standard sulphate-s solution</u>: 0.443 grams of sodium sulphate ($Na_2SO_4.10H_2O$), were dissolved in distilled water 1 litre, which gives the concentration 100 µg SO_4^{2-} - S ml^{-1}.

4.2.5 Determination of dry weight:

Soil samples were dried overnight at 60^0C and the dry weight was determined by weighing, using the sensitive electrical balance.

4.2.6 Determination of pH:

The pH of the soil solutions were determined by using a glass electrode pH meter.

4.3 RESULTS AND DISCUSSION

4.3.1 Effect of silicon compounds on sulphur oxidation in agricultural loam soil

The effect of increasing amounts of silicic acid and rock potash on concentrations of water-soluble sulphate in the agricultural loam soil, over a 14 day incubation period, is shown in Fig 4.2.

Sulphate concentrations, reflecting sulphur oxidation, generally increased with increasing amounts of both silicon compounds, with silicic acid having the most pronounced stimulatory effect.

Essentially the same trend was observed when ($LiCl_2$, 0.1M) was used as the extracting agent. In this case, sodium silicate was also added; this proved to have the most marked stimulatory effect of the three silicon compounds used (Fig. 4.3).

Changes in sulphate concentration ($LiCl_2$,0.5M) in the agricultural loam soil following amendment with silicic acid and sodium and potassium silicate over a 28 day incubation period are shown in Fig. 4.4. In the case of silicic acid the previously observed trend of increasing sulphate concentration following amendment is generally seen over the entire 28 day incubation; and with the exception of the 21 day sample, this observed increase in sulphate concentration correlates with increasing amount of added silicon. (Fig. 4.4 a). Increased sulphate concentrations were seen following amendment at all concentrations of added sodium silicate for the first 14 days, after which time there is a tendency for the reverse to occur, and sulphate concentrations lower than the control result (Fig. 4.4 b). The same trend is broadly seen following sulphur amendment with sodium and potassium silicates (Fig. 4.4 b, c).

The results show that the silicon compounds used here stimulate sulphur oxidation in soils, leading to increased sulphate concentrations, at least during the first 14 days of the incubation, period, with sulphate concentrations increasing with increasing amount of added silicon compound. After 14 days, with the exception of silicic acid, sulphate concentrations fall below the control value. The reasons for the initial stimulation in sulphur oxidation are unclear, although since they occur with all three compounds used, and not just sodium silicate, the effect is unlikely to be due solely to increases in soil pH.

An interesting subsidiary point arises from these results, namely how long should arbitrary based soil incubation studies be conducted.

Clearly, had the above experiment been terminated at day 14 of the incubation period, the reported effect would be that silicon compound stimulate sulphur oxidation in this soil. However, the reverse is true if we consider results obtained from days 14-28 of the incubation period. These observations beg the question, what would the trend have been over the following 28 days had the incubation experiment been continued. Clearly the length of the incubation used in such experiments is a matter of accepted practice, rather than being a particularly meaningful period.

FIG: 4.2

Effect of different amounts (1, 5, 10, and 20 g), of silicic acid and rock potash on sulphur oxidation in agricultural loam soil 14-day incubation (Water extracted).

(Means of triplicate, ± standard error).

SO_4^{2-} - S µg (g^{-1} dry soil)

——▲—— soil containing added silicic acid

——■—— soil containing added rock potash

FIG: 4 . 2

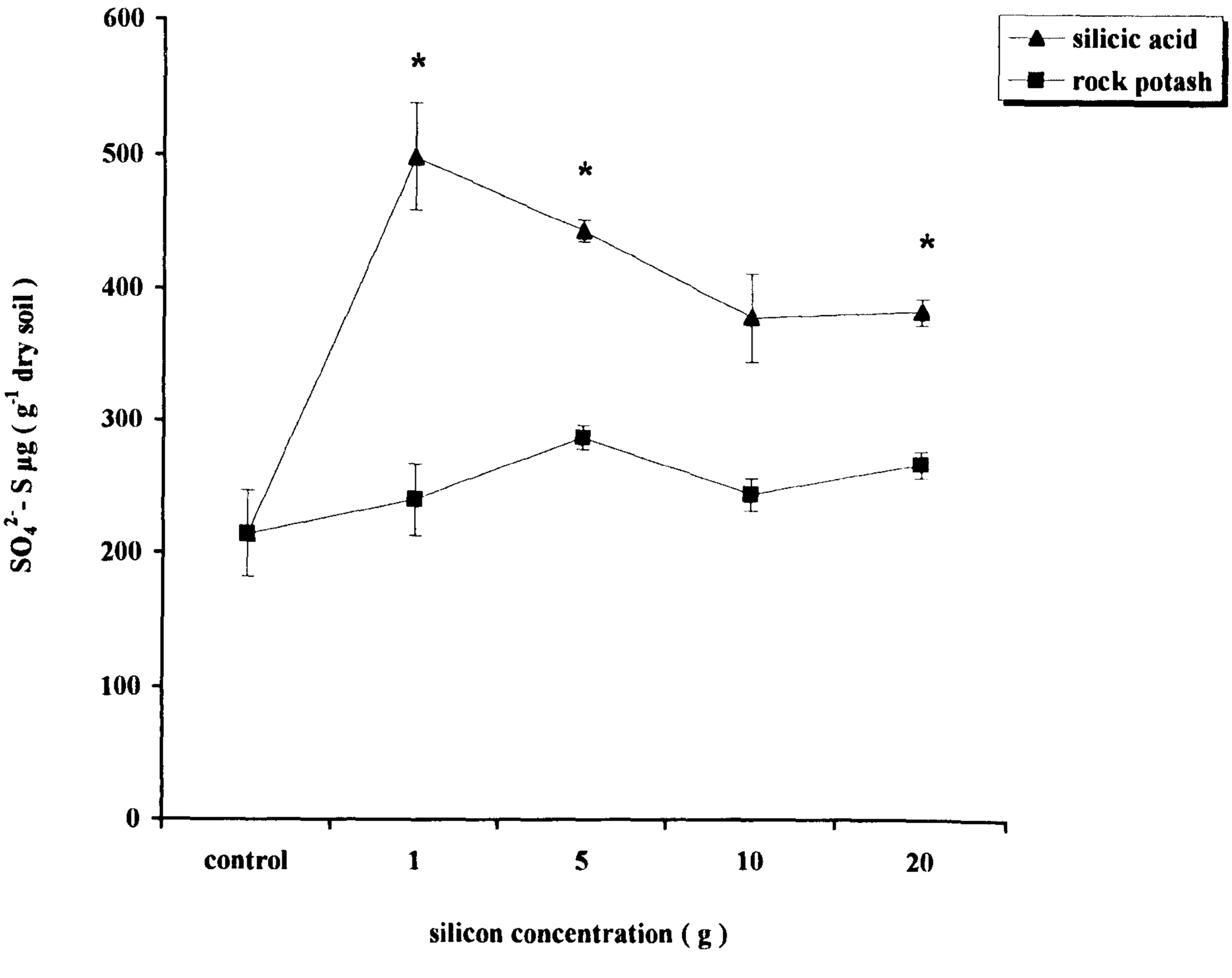

600
500
400
300
200
100
0
SO_4^{2-} - S µg (g^{-1} dry soil)
control
1
5
10
20
silicic acid
rock potash
*
*
*
silicon concentration (g)

FIG: 4.3

Effect of different amounts (1, 5, 10, and 20 g), of added silicic acid, sodium silicate and potassium silicate on sulphur oxidation in agricultural loam soil, 14-day incubation. ($LiCl_2$, 0.1M)

——▲—— silicic acid

——●—— sodium silicate

——♦—— potassium silicate

Means of triplicate, ± standard error. * Significant difference from control, $P < 0.05$.

FIG: 4.3

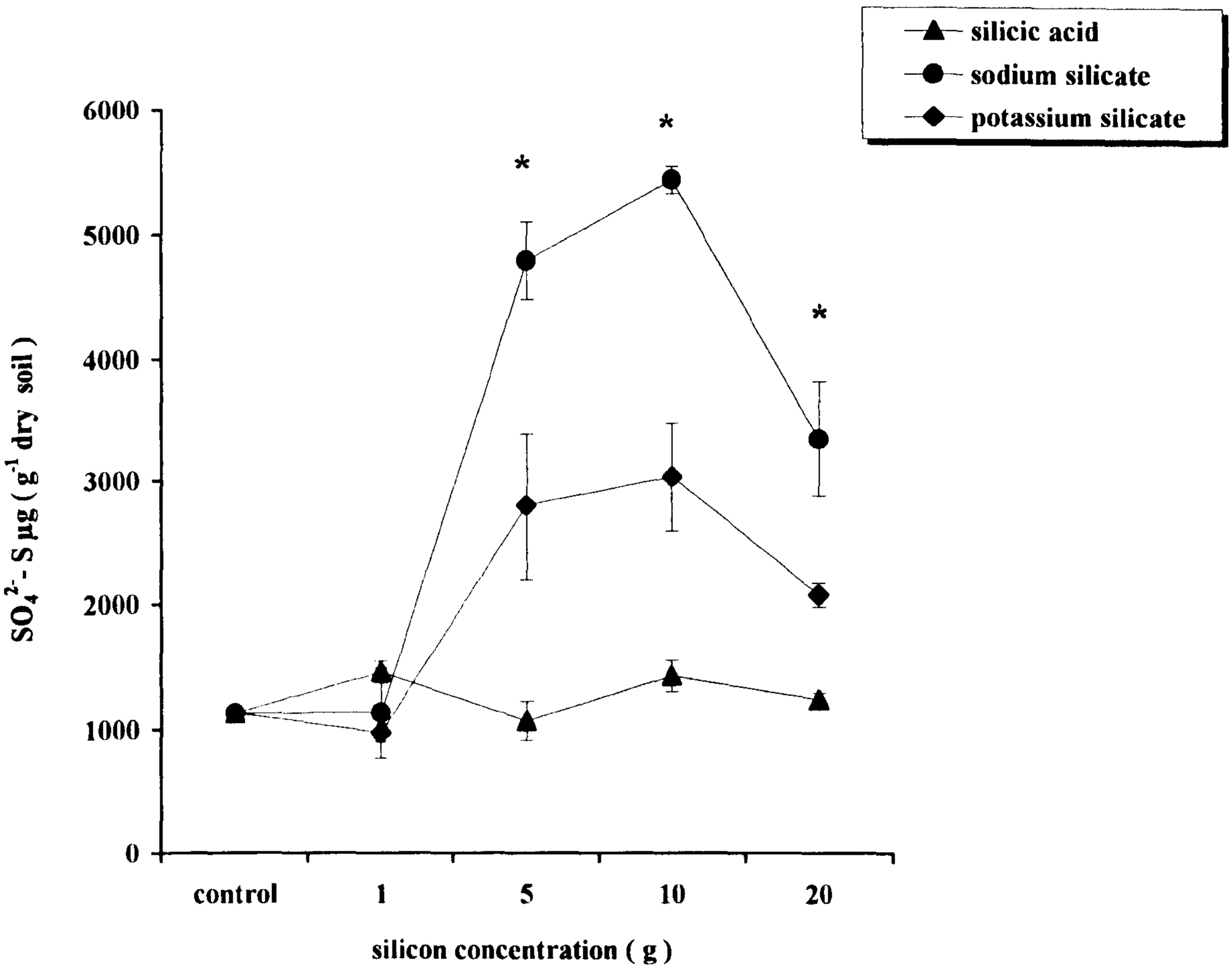

silicic acid
sodium silicate
potassium silicate
SO_4^{2-} - S µg (g^{-1} dry soil)
6000
5000
4000
3000
2000
1000
0
*
*
*
control
1
5
10
20
silicon concentration (g)

FIG: 4.4

Effect of different amounts (1, 5, 10, and 20 g), of added silicic acid, sodium silicate and potassium silicate on sulphur oxidation in agriculture soil. The experiment was carried out on 7, 14, 21 and 28^{th} day using 0.5 M $LiCl_2$ extract.

SO_4^{2-} - S μg (g^{-1} dry soil)

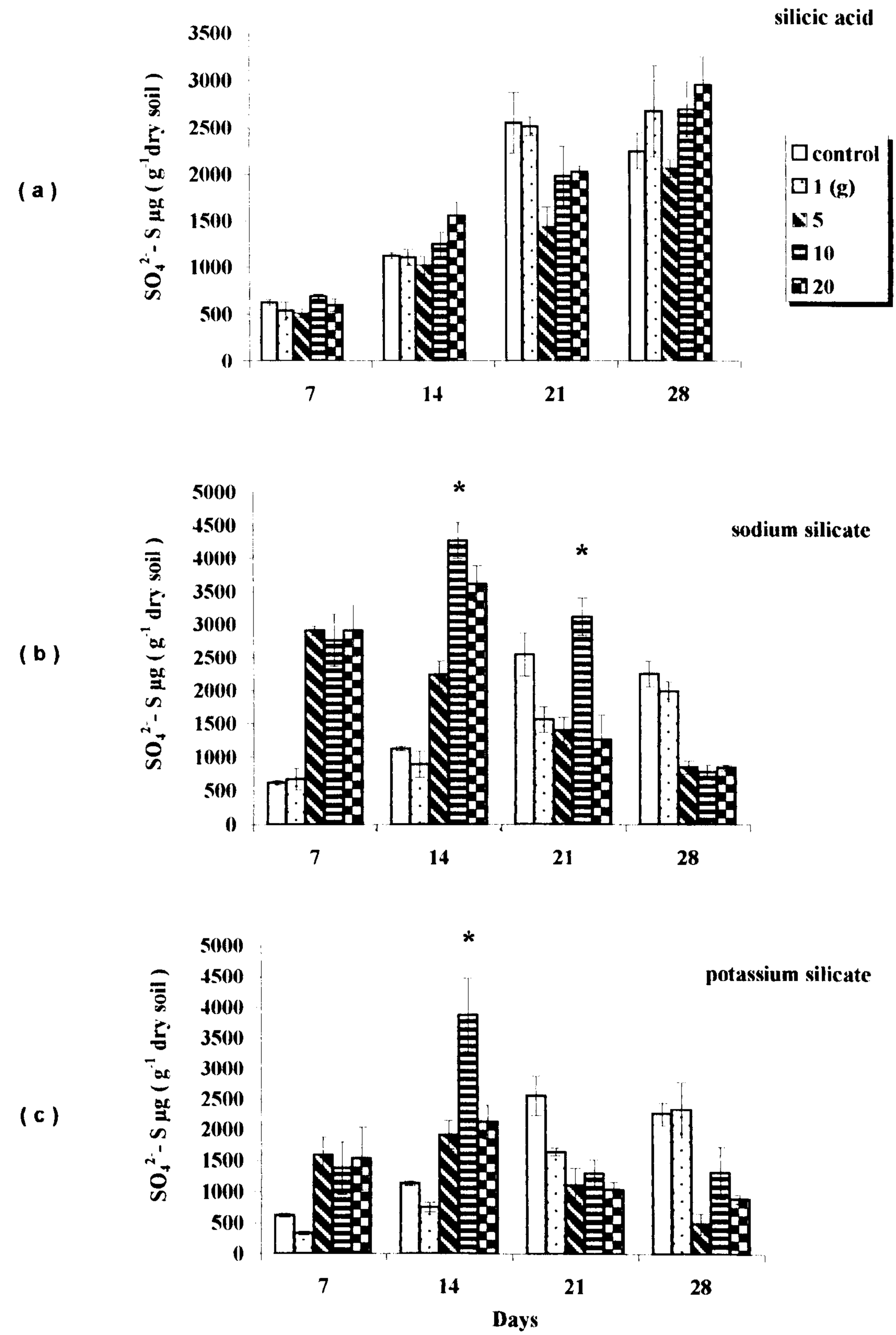
(a) soil containing silicic acid

(b) soil containing sodium silicate

(c) soil containing potassium silicate.

Means of triplicate, ± standard error. * Significant difference from control, $P<0.05$.

FIG: 4.4


(a)
silicic acid
control
1 (g)
5
10
20
SO_4^{2-} - S µg (g^{-1} dry soil)
3500
3000
2500
2000
1500
1000
500
0
7
14
21
28
(b)
sodium silicate
*
*
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
(c)
potassium silicate
*
Days

CHAPTER FIVE

EFFECT OF SILICON ON SOIL- ENZYMES, SOIL - MICROBIAL RESPIRATION AND BIOMASS

CHAPTER 5- (a) ENZYME ACTIVITY IN SOIL FOLLOWING AMENDMENT WITH SILICIC ACID

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5.1 INTRODUCTION.

As plant roots and soil organisms grow, multiply, and then proceed through the process of cell destruction, they release many biologically important substances such as enzymes, vitamins, amino acids, sugars, antibiotics etc. into the soil medium. Enzymes are proteinic substances (catalysts), which, without undergoing permanent changes, accelerate chemical reactions from 10^6 to 10^{12} times those of uncatalysed reactions. Soil enzymes and micro-organisms play a major role in altering the constituents of soil organic matter to other simpler organic and inorganic molecules, and bring the nutrient cycle in soil. .

The availability of extracellular enzymes in soil was, reported for the first time by Wood (1899). Since then, the main problem in studying the soil enzymes was to separate the activities of microorganisms from the activities of extacellular enzymes (Burns, 1978). After 1950, studies on soil enzymes really progressed with the improved understanding of the enzymatic reactions and application of various methods which became available from plant, animal and microbial biochemistry (Burns, 1978). Soil enzymes are primarily produced from microbial biomass, but they can also be originated from plants and animal residues (Burns, 1978). Enzyme activities are related to soil properties and they can be used as criterion of soil properties and fertility. In soil, enzyme activity could be result of both accumulated enzymes and those released by micro-organisms. Burns (1978) has suggested the function of accumulated enzymes in the soil and concluded that they have a

role in (a) the first phase of decomposition of organic residues and in (b) the transformation of some mineral substances even under conditions unsuitable for microbial proliferation. The accumulated enzymes are active soil enzymes which are not formed by micro-organisms. Enzyme activities in soil are largely due to free enzymes, such as exoenzymes released from living cells, endoenzymes released from disintegrating cells, and enzymes bound to cell constituents. Proliferating microorganisms produce enzymes that are released to the soil, or that remain within the multiplying cells. Different enzymes are specific for different types of chemical reactions in which they are involved. The activity of the soil enzymes (e.g. alkaline phosphatase, acid phosphatase, arylsulphatase, amidase, dehydrogenase, invertase, urease etc). involved in carbon, nitrogen, phosphorus, and sulphur cycling increased in a soil containing organic matter by an average of two to four fold by incorporation of the four organic amendments when compared with the unamended soil during the thirty one month study and the straw amendment was the most effective means of elevating the soil enzyme activity (Martens *et al.*, 1992).

Enzymes being proteinic in nature, can be denatured by increased temperatures and higher pH. Physical and chemical states of enzymes and their effects on chemical reactions, largely depend on ionic strength, temperature and pH. The changes in environmental, agronomic and climatic conditions, influence the microbial activities and magnitude of several enzymatic reactions. Conrad (1942) mentioned that cropping and cultural practices that added organic matter to the soil resulted in higher urease activities. Practices which cause organic matter depletion, result in a lower enzymatic activity. Rogers *et al.* (1941), attributed the hydrolysis of glycerophosphate, nucleic acid, and nucleotides to the action of exoenzymes produced by growing corn roots. These enzymes were found in a gel-like material adhering to root surfaces.

Mortland and Gieseking (1952) concluded that clays (e.g. montmorillonite, illite, kaolinite etc.) inhibit the enzymatic hydrolysis of organic phosphorus compounds. The amount of inhibition was proportional to the cation-exchange capacity of the clay. They suggested that the inhibition was probably caused by adsorption of the enzyme by the clay. Enzymes released into the soil medium, microorganisms, and substrate molecules probably exist in an adsorbed form on the surfaces of minerals.

I was interested to determine the effects of the silicic acid on the microbial and enzyme (arylsulphatase and dehydrogenase) activity of an agricultural loamy soil.

5.1.1 Arylsulphatase

Sulphur, particularly in the form of sulphate (SO_4^{2-}) is an essential nutrient for most of the organisms. Almost all sulphur available in soils is in organic combinations, with two fractions accounting for most of the organic sulphur present (Freney, 1967). As studies have shown that up to 90% of soil sulphur is in the form of organic sulphate esters (R-C-O-SO_3^-), mineralisation of ester sulphates by the enzyme (sulphatase), in soil is an important process. In sulphur deficient soils, sulphatases, play an important role by releasing the sulphur from the organic compounds and thereby making sulphate available to plants which in turn make the soil more fertile from the agricultural point of view.

Sulphatases hydrolyse sulphuric acid esters and catalyse the reaction as:

$$R - C - O - SO_3^- + H_2O \longrightarrow R - C - OH + H^+ + SO_4^{2-}$$

Sulphatases are classified, on the basis of organic sulphate esters type, which is hydrolysed by the action of these enzymes. The main recognized groups included arylsulphatases, alkylsulphatases, steroid sulphatases, chondrosulphatases glucosulphatases, and mycosulphatases (Roy, 1960). Arylsulphohydrolases, that are also termed as "phenolsulphatases" or "arylsulphatases"; aryl-sulphate sulphohydrolase). The enzymes that fit this category were initially observed by reason of their arylsulphatase activity, they have had and are now receiving different designations. In man, the purified arylsulphatase A is also a cerebroside sulphatase , and arylsulphatase B is involved with desulphation of *N*-acetylgalactosamine 4-sulphate residues in the metabolism of glycosaminoglycan. Sulphatase C, a membrane-bound protein, is now considered as the

enzyme physiologically responsible for the hydrolysis of sterols esterified to sulphate (steryl sulphatase).

Arysulphatases are ubiquitous in distribution. These enzymes are present in plants animals, humans and micro-organisms and are often released into soil, by plants and microbes. Tabatabai and Bremner (1970) first reported that the availability of arylsulphatases in soil and they mentioned that the organic soil sulphur mineralisation to SO_4^{2-}, is the result of arylsulphatases and other sulphatases that have a major role in soil processes. Since then, several studies have been made on arylsulphatases in different soils (Cooper, 1972; Speir and Ross, 1975). These enzymes were also found in marine, lake sediment (Chandramohan *et al.*, 1974; King and Klug, 1980), in salt marsh soils (Oshrain and Wiebe, 1979; Wainwright, 1981) and in intertidal sands (Wainwright, 1981).

5.1.2 Dehydrogenase

Dehydrogenases are enzymes that remove electrons and hydrogen ions from reduced substrates have NAD^+ or $NADP^+$ as their coenzyme. NAD^+ can exist in a reduced form, $NADH + H^+$, to form an O/R system.

The microbial oxidation of organic substances under aerobic conditions is linked to a membrane-bound electron transfer chain with O_2 as a final electron acceptor. The electron transport system is coupled with the synthesis of ATP, which is called "oxidative phosphorylation". Electrons are collected in NADH, from different substrates through the action of NAD-linked dehydrogenases. These electrons funnel into the chain via the flavoprotein NADH dehydrogenase. Other respiratory substrates are dehydrogenated by flavin-linked dehydrogenases, such as succinate dehydrogenase and acyl-CoA dehydrogenase, which funnel electrons into the chain via ubiquinone.

NAD^+ and ubiquinone, thus work to collect reducing equivalents from respiratory substrates oxidised by pyridine-linked and flavin-linked dehydrogenases. The electrons are further transferred to the cytochrome system, where they are oxidised by O_2.

Skujins, (1976) mentioned the activity of dehydrogenase in soil which gives correlative information on the biological activity and microbial populations in soil and the biochemical properties of these enzymes suggest that the availability of free dehydrogenases are unlikely in soil. Dehydrogenase activity is considered to reflect the total range of oxidative activities of the soil micro-organisms (Ladd, 1978). Stevenson, (1959) and Skujins, (1973) have reported that dehydrogenase activity in soil does not appear to correlate with the numbers of soil micro-organisms, but does correlate well with O_2 uptake and CO_2 release from soil. However in contrast, many scientists have failed to

find a correlation between dehydrogenase activity and respiration (Ross and Roberte, 1970; Skujins, 1976). Dehydrogenase activities can be increased with increasing microbial populations in soil amended with nutrients (Ladd and Paul, 1973). It is suggested that soil water content and temperature influence the dehydrogenase activity by affecting the soil oxidation – reduction status (Brzezinska *et al.*, 1998).

5. (b) EFFECT OF SILICON ON MICROBIAL-RESPIRATION AND BIOMASS IN AGRICULTURAL SOIL

****

5.2 INTRODUCTION

Microbial respiration in soil, was one of the most used indexes of the microbial activity of soil in the past and is still today. Wollny (1881) discussed the production of CO_2 as a measure of microbial activity of soils. Since then the measurements of microbial respiration in soil has been studied by Russell (1905), Neller (1922), Waksman and Starkey (1924) and Stotzky and Norman, (1961a). Measurements of respiration are known to be well correlated with factors other than microbial activity, such as organic matter content, nitrogen or phosphorus transformations, metabolic intermediates, pH, average microbial numbers, and changes in soil weight (Stotzky, 1960). Macfadyen, (1973), has reported that many ecological studies involve estimations of biological activity through measurements of O_2 uptake or CO_2 release from mineral soil.

The soil microflora play an important role in capturing energy and carbon for their cell synthesis. The dry weight of plant and animal tissues contains approximately 45 to 50% of carbon. After microbial metabolization of animal and plant tissues, O_2 is consumed and CO_2 is released. The combined metabolism of mixed populations of microorganisms in complex substrates such as soils, natural surface waters, or decaying organic materials can be quantitatively determined by measurement of total respiration. Fungi and actinomycetes can convert substrate carbon to cell carbon more efficiently than the aerobic bacteria and

anaerobic bacteria can utilize carbohydrates inefficiently. (Alexander, 1977), However heterotrophs are regarded as being involved in organic matter degradation.

The decomposition of native organic matter (humus) reflects the biological availability of soil carbon while the release of CO_2 following the addition of relatively simple substrates is an estimation of the biodegradability of the test compound (Alexander, 1977). Carbon production increases on the additions of organic materials to the soil. Soil respiration has also been studied in various soils amendment experiments, in which the most common additive being glucose. Anderson and Domsch (1974, 1975) observed that bacterial and fungal respiration was stimulated within minutes into maximal activity in short-term (1-6 h) experiments by the addition of saturating quantities of a readily-available substrate such as glucose. The increased rate of respiration remains stable for up to 6-8 h, after which time it began to increase. The further increase after 6-8 h was attributed to cell division and population growth. Various environmental factors are involved in humus decomposition including organic matter level of the soil, cultivation, temperature moisture, pH, depth, and aeration (Alexander, 1977).

Non-biological CO_2 formation or consumption of O_2 can interfere with respiration measurements. CO_2 may be produced by chemical decarboxylation (Bunt and Rovira, 1955), by cell-free, heat-stable enzymes (Bunt and Rovira, 1955), or by the action on free soil carbonates of added chemicals or organic acids produced during metabolism (Chase and Gray, 1957).

Microbial biomass is defined as the part of the organic matter in soil that constitutes living micro-organisms smaller than 5-10 μm^3. In recent years great interest has developed concerning the size of the soil microbial biomass. This is because the biomass represents the "eye of the needle" through which all soil organic matter must eventually pass. Soil

microbial biomass plays a major role in soil fertility and the processes of soil formation. Biomass carbon is expressed in milligrams of carbon kg^{-1} soil or micrograms of carbon g^{-1} dry weight, it ranges from 1 to 5% of soil organic matter (Jenkinson and Ladd 1981; Sparling 1985; Smith and Paul 1990). Measurements of soil microbial biomass have been used in studies of the flow of carbon, cycling of nutrients, and plant productivity in a variety of terrestrial ecosystems. They provide a measure of the quantity of living microbial biomass present in the soil at a particular point in time, i.e., the "standing crop". The data can be used for assessing changes in soil organic matter caused by soil management (Powlson *et al.*, 1987) and tillage practices (Carter 1986), for assessing the impact of management on soil strength and porosity, soil structure and aggregate stability, for estimating seasonal fluctuations in microbial biomass (Ross 1990), and for serving as an indicator of the presence of toxins, e.g., metal toxicity (Brookes *et al.* 1986). In addition measurements of the carbon and nutrients contained in the microbial biomass provide a basis for studies of the formation and turnover of soil organic matter, as the microbial biomass is one of the key definable fractions.

Several methods have been used to determine microbial biomass in soil. Currently available methods include:

(a) Direct counting of variously stained organisms (Nicholas and Parkinson, 1967; Ingham and Klein, 1984).

(b) Analysis of soil extracts for enzymes (Skujins, 1967; Kiss *et al.*, 1975), cell wall components (Millar, 1970; West, 1986) or living cell components (Verstraeten *et al.*, 1983).

(c) Measurements of substrate-induced respiration (SIR; Anderson and Domsch, 1975, 1978; West *et al.*, 1986) or ammonification (Alef *et al.*, 1988).

(d) Determinations of $CHCl_3$-labile (microbially bound) elements (Jenkinson, 1966; Jenkinson and Powlson, 1976; Brookes *et al.*, 1985; Vance *et al.*, 1987).

Each of the procedures listed above have particular advantages and limitations which depend on the nature of the research questions and the microbial habitats investigated (Anderson and Domsch, 1978). Of these substrate-induced respiration (SIR) and fumigation-incubation (FI) are two basic methods of biomass determination.

The chloroform fumigation-incubation method (CFIM) is generally thought to be the most useful for soil studies. In the CFIM, microorganisms are made susceptible to mineralization by fumigation with $CHCl_3$ vapour. This disrupts the microbial cell membranes and releases internal constituents to the microorganisms surviving $CHCl_3$ treatments or added inoculum. Jenkinson and Ladd, (1981), have argued that sufficient organisms survive fumigation to make the use of an inoculum unnecessary. The proportion of biomass C mineralised during the 10 days after $CHCl_3$ fumigation (kc) has been derived experimentally determining the mineralization rate of known quantities of microbial C in fumigated soil. Jenkinson (1966) proposed a value of 0.30 for kc that was later revised to 0.5 (=50% mineralization) based on a greater number and wider variety of organisms (Jenkinson, 1976). Adams and Laughlin (1981) reported a value of 0.55 for kc based on 10 different organisms including both bacteria and fungi. Anderson and Domsch (1978), used an incubation temperature of 22^0C and used12 (radio-labelled) species of bacteria and 15 (radio-labelled) species of fungi added to 4 different soils suggested a k-factor of 0.411(=41.1% mineralization).

The chloroform fumigation-incubation technique (Jenkinson and Powlson, 1976; Anderson and Domsch, 1978) has been used extensively but there are problems using it for acid soil or soil which have recently had organic amendments.

The aim of the following experiments was to determine the effects of silicon compounds, on soil-microbial respiration following glucose amendment and on biomass treated with alcohol free-chloroform fumigation.

5.3 MATERIALS AND METHODS

5.3.1 Effect of various amounts of silicic acid on the activity of "arylsulphatase" in Agricultural soil

Agricultural loam soil was collected (10 cm depth) from Sheffield, and divided into two groups

(a) Soil amended with silicic acid.

(b) Soil lacking added silicic acid.

To triplicate fresh agricultural loam soil samples (100 g, sieved 2mm, roots and animals free soil) containing in polythene plastic bags were added, 1, 5, 10, and 20 grams silicic acid and mixed. The other soil set was lacking added silicic acid. 5 mls deionized (sterile) water were sprinkled, mixed thoroughly and the samples were incubated at 25^0 C for 14 days.

Measurement of enzyme-activity was made on 0, 7, 14, 21, and 28^{th} day. Control, was set-up without adding *p*-nitrophenyl sulphate solution and was compared with each sample of treatment.

5.3.1.1 Measurement of arylsulphatase activity in soil

To soil (1g), contained in universal vials (25 ml), 4 ml acetate buffer, 0.25 ml toluene and 1 ml *p*-nitrophenyl sulphate solution were pipetted, swirled for few seconds to mix the contents, screwed on top and placed in a water bath at 25^0 C. After 1 hr vials were removed from the water bath, and 1ml $CaCl_2$ (0.5 M), 4 ml NaOH (0.5 M) were added,

vials were swirled for few seconds, and the soil suspension was filtered through Whatman No: 1 folded filter paper into a test tube.

Control was performed with each soil sample analyzed to allow for colour not derived from *p*-nitrophenol released by arylsulphatase activity. For control, above procedure was followed except 1 ml of *p*-nitrophenyl sulphate solution. After incubation at 25^0 C for 1 hr, 1 ml of $CaCl_2$ (0.5 M) and 4 ml of NaOH (0.5 M) were added and then 1 ml of *p*-nitrophenyl sulphate solution was added immediately before filtration of the soil suspension into a test tube. Filtrate was transferred to a 4 ml cuvette and intensity of the yellow colour was measured at 400 nm in the spectrophotometer against a reagent blank.

5.3.2 Effect of various amounts of silicic acid on the activity of "dehydrogenase" in Agricultural soil.

Fresh agricultural soil was divided into two groups as mentioned before.

To triplicate soil samples (100 g, sieved 2 mm, roots and animals free soil), were added 1, 5, 10 and 20 grams of silicic acid and mixed in polythene bags plus 5 ml deionised water (autoclaved) was added and mixed thoroughly. Control was set-up lacking added silicic acid in soil. All plastic bags were closed with rubber bands, leaving small holes for air, and incubated at 25^0 C for two weeks. Dehydrogenase activity was determined every week by using the following method:

5.3.2.1 Preparation of chemicals

(a) Tris-HCl buffer (100 mM)

12.1 gram of Tris (hydroxy methyl) aminomethane were dissolved in 700 ml distilled water, pH was adjusted to 7.6 (pH of the soil was 7.3, between the pH range of 6 to 7.5) and added more distilled water up to 1000 ml.

(b) Triphenyltetrazolium chloride (TTC) solution

1g of triphenyltetrazolium chloride (TTC) was dissolved in 80 ml tris-buffer and volume was made up with the same buffer to 100 ml.

(c) Extractant

Acetone (analytical grade)

TPF standard solution

50 mg of Triphenyl formazan (TPF) were dissolved in 80 ml of acetone (500µg TPF ml^{-1}) and the volume was made up to 100 ml with acetone. Field-moist agricultural soil (5 g) was weighed into 60 ml glass bottles and mixed with 5 ml triphenyltetrazolium chloride (TTC) solution. All the bottles were sealed with solvent resistant-rubber stoppers and incubated for 24 hours at 30^0 C. The control contains only 5 ml tris-buffer (without TTC). After the incubation, 40 ml acetone was added to each bottle and the bottles were shaken thoroughly and further incubated at room temperature for 2 hours in the dark (shaking the tubes at intervals). The soil suspension (15 ml) was then filtered through Whatman No.1 folded filter paper and the optical density of the clear supernatant was measured against the blank at 546 nm.

5.3.2.2 Calibration curve

TPF standard solution, was pipetted as 0, 0.5, 1.0, 2.0, 3.0 and 4.0 ml in a volumetric flask (50 ml), 8.3 ml Tris buffer (pH 7.6) was then added and the volume was brought up with acetone to 50 ml mark to obtain the concentrations of 0,5, 10, 20, 30, and 40 μg TPF ml^{-1}.

5.3.3 Effect of various amounts of silicic acid and sodium silicate on the production of carbon dioxide (CO_2), from agricultural soil

Agricultural soil was collected by digging 10 cm deep from the soil surface. **Anderson and Domsch's glucose-amendment technique** was used with some modifications.

To triplicate samples of fresh agricultural soil (100g, sieved 2mm, roots free soil) in Kilner's jars, were added, 0.1, 0.5, 1.0, 5.0 and 10 g silicic acid and sodium silicate (each silicon compound per triplicate set of soil), plus, 8 ml of 1% glucose solution were sprinkled and mixed thoroughly. Controls were set-up lacking added silicon compounds. All the samples were incubated overnight at 25^0C. After incubation, potassium hydroxide (60 ml, 0.1M KOH) in 100 ml glass beakers were placed in the Kilner jars, and the jars were then sealed and incubated at 25^0 C for 4, 6, 8 and 10 days. CO_2 released, was measured by applying the acid-base titration method.

5.3.3.1 Titration-method

After incubation, KOH (10 ml, 0.1M) solution were transferred to 100 ml Erlenmeyer flask. Into the Erlenmeyer flask 2 ml $BaCl_2$ solution (0.5 M) were added. This is used to precipitate the potassium carbonate to prevent over-estimation of the titration end-point. Phenolphthalein indicator (3 drops) were added and the solution was titrated with 0.1M HCl to end-point by using 25 ml burette. Blanks were also titrated of 10 ml fresh KOH.

The data were calculated thus:

5.3.3.2 Calculations

Where vol: of KOH neutralized x 0.022 = wt of CO_2 absorbed and converted to $K_2 CO_3$.

OR 1 ml of 0.1 M HCL ≡ 2.2 mg CO_2

5.3.4 Determination of soil microbial biomass by chloroform-fumigation (CF) technique in agricultural soil, following amendment with silicic acid and sodium silicate.

To triplicate fresh agricultural soil samples (100 g, sieved 2 mm, roots and animals free) in polythene bags, were added, 1.0 and 5.0 g silicic acid and sodium silicate (each silicon compound per soil set) plus 8 ml deionised (sterile) water was sprinkled over the soil sample and mixed thoroughly. All the polythene bags were closed with rubber band, leaving a small hole for air and incubated at 25^0 C for 14 days.

After incubation, the soil was placed in Kilner jars. Control was set-up as, soil (fumigated) lacking added silicon compounds.

The Kilner jars containing soil samples, were labeled and placed at the center of the fumigator along with a beaker containing chloroform (ethanol-free) (100 ml) and the fumigator was evacuated for 2 minutes and then sealed, using silicone grease and parafilm. Vacuum was created with vacuum pump at 760 mm/ Hg.

Maintaining the vacuum, left for 3 hours (in this time, the chloroform, was boiled which diffused into the soil). After removing the lid, the fumigator was evacuated (5 times) for 10 minutes period, opening between evacuations. After removing the chloroform from soil containing in Kilner jars, a beaker containing (60 ml, KOH 0.1M) was placed and sealed tightly. All the jars were incubated at 25^0 C for 1 week.

5.3.4.1 Chloroform (ethanol-free $CHCl_3$)

Commercial chloroform was washed with about 5% by volume H_2SO_4 (conc:) by shaking in a separating funnel to separate off the acid, and re-washed with 10 rinses of distilled water. (ethanol-free $CHCl_3$ was stored in the dark to prevent photochemical build-up of explosive-by products). Ethanol-free $CHCl_3$ is recommended because ethanol cannot be completely removed from soil after fumigation (Jenikinson 1988) and it is used as a substrate, mineralized to CO_2, and thus is incorrectly measured as biomass C.

The CO_2 evolved during the incubation was measured by the Titration method, as described above.

5.3.4.2 Calculations

Where vol: of KOH neutralized x 0.022 = wt of CO_2 absorbed and converted to $K_2 CO_3$.

OR 1 ml of 0.1 M HCL ≡ 2.2 mg CO_2

The CO_2 evolved during the incubation results from mineralization of the dead microbial biomass was measured by the following formula:

B= F/Kc

Where B= Biomass carbon

F= CO_2-C evolved from fumigated soil over 10 days

Kc= portion of biomass carbon mineralized to CO_2 (0.411 = 41.1% mineralization).

5.4 RESULTS AND DISCUSSION

5.4.1 Effect of silicic acid addition on soil arylsulphatase activity

The effect of silicic acid on the activity of this soil enzyme can be quickly summarized:

Silicic acid had an inhibitory effect on soil arylsulphatase activity in the agricultural loam soil over the full 28 day incubation period, with inhibition increasing with increasing concentration of added silicic acid (Fig. 5.1).

The effect of silicic acid on this enzyme was studied because it is considered to be an essential enzyme in relation to the release of the sulphate ion from soil organic sulphates, and is therefore an important factor in relation to soil fertility. Soil enzymes can be measured in relation to enzyme activity at any one point (i.e. in the presence of an inhibitor, like toluene) or enzyme synthesis over a time period, without an inhibitor of microbial activity being added. The results presented here refer to the former, i.e. they show that silicic acid inhibits arylsulphatase in the soil derived from a number of sources, including microorganisms, plants and a soil micro-fauna.

The results suggest that in this respect at least, silicic acid addition would have a negative impact on soil fertility.

5.4.2 Effect of silicic acid on dehydrogenase activity in the agricultural loam soil

Essentially the same result was seen above when the effect of silicic acid on dehydrogenase activity was measured, i.e. silicic acid inhibited the activity of this enzyme (over a 14 day incubation period) with inhibition increasing with increasing concentration of added silicic acid (Fig 5.2). Since dehydrogenase activity is usually considered to be a measure of microbial activity in soil, these results suggest that silicic acid has a detrimental impact on the soil microflora. It is noteworthy however, that while dehydrogenase activity was inhibited, the addition of silicic acid to the soil stimulated the numbers of total, aerobic heterotrophic bacteria, as measured by the dilution plate count (Fig 2.11 d). This suggests that two parameters are not directly comparable. Indeed there is considerable debate as to whether a good correlation exists between dehydrogenase activity and soil bacterial numbers, as determined by plate counting.

5.4.3 Effect of silicic acid and sodium silicate on soil respiration in the agricultural loam soil.

Silicic acid had only a marginal effect on respiration, with a tendency to cause slight decreases in CO_2 release over the 10 day incubation period (Fig 5.3 a). Such a decrease in respiration, correlates approximately to the above observed inhibition in dehydrogenase activity.

In contrast to silicic acid, the addition of sodium silicate led to a marked stimulation in the release of CO_2 from the agricultural loam, at the concentration of 5 g of added

sodium silicate (Fig. 5.3 b). This marked effect is again associated with an increase in soil pH (data deduced from other experiments).

5.4.4 Effect of silicic acid and sodium silicate on biomass in the agricultural loam soil.

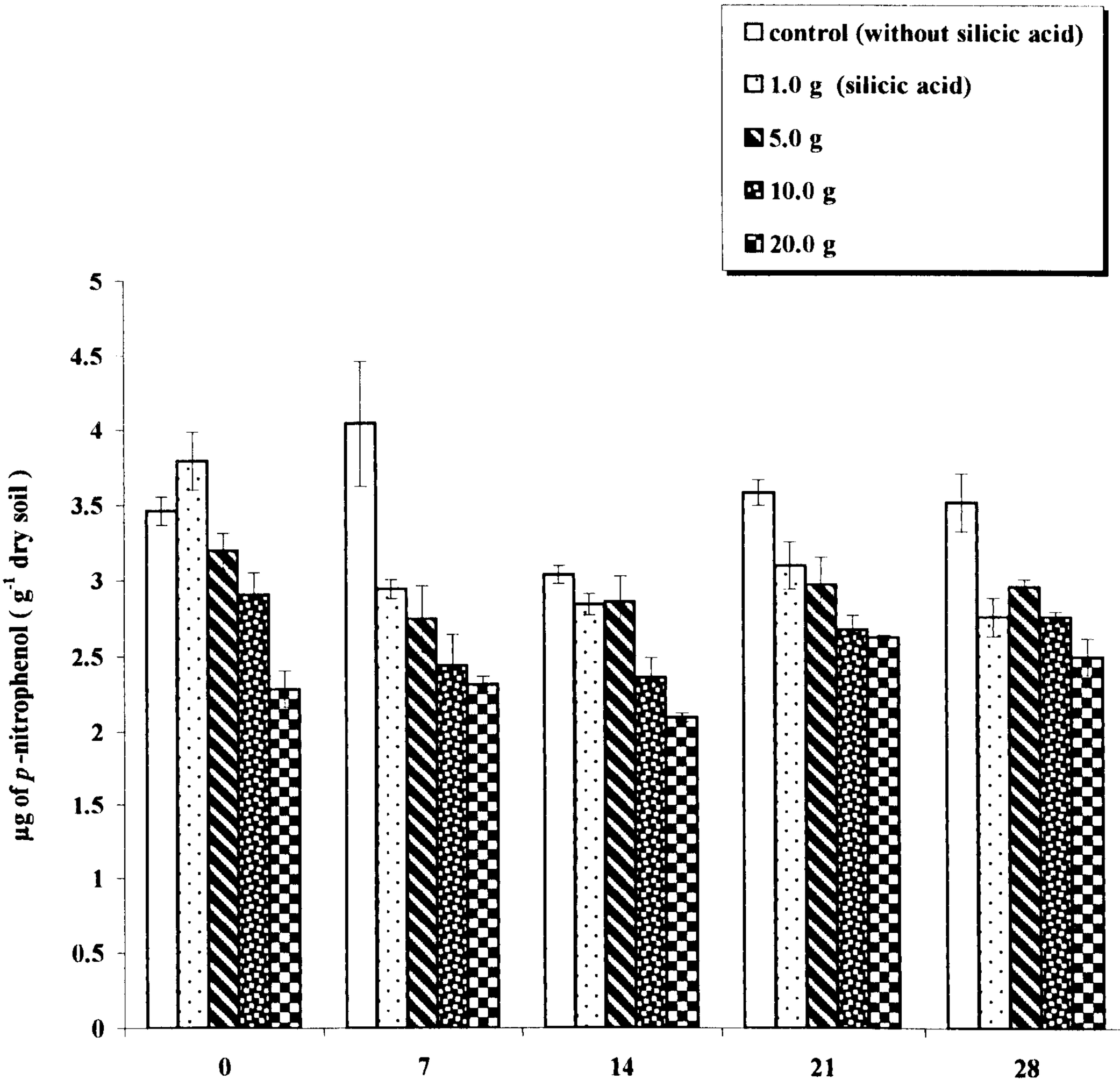
The above pattern seen with respiration was again repeated with soil biomass, i. e., while silicic acid addition led to reduction in soil biomass, sodium silicate addition resulted in a marked stimulation (Fig 5.4). Again, this effect can be attributed to an increase in soil pH following the addition of sodium silicate stimulating numbers of soil microorganisms and, as a result, biomass.

FIG: 5.1

Effect of different amounts of added silicic acid on the activity of "arylsulphatase" in agricultural soil. (Means of triplicate, ± standard error).

µg of *p*-nitrophenol (g^{-1} dry weight $soil^{-1}$)

FIG: 5.1

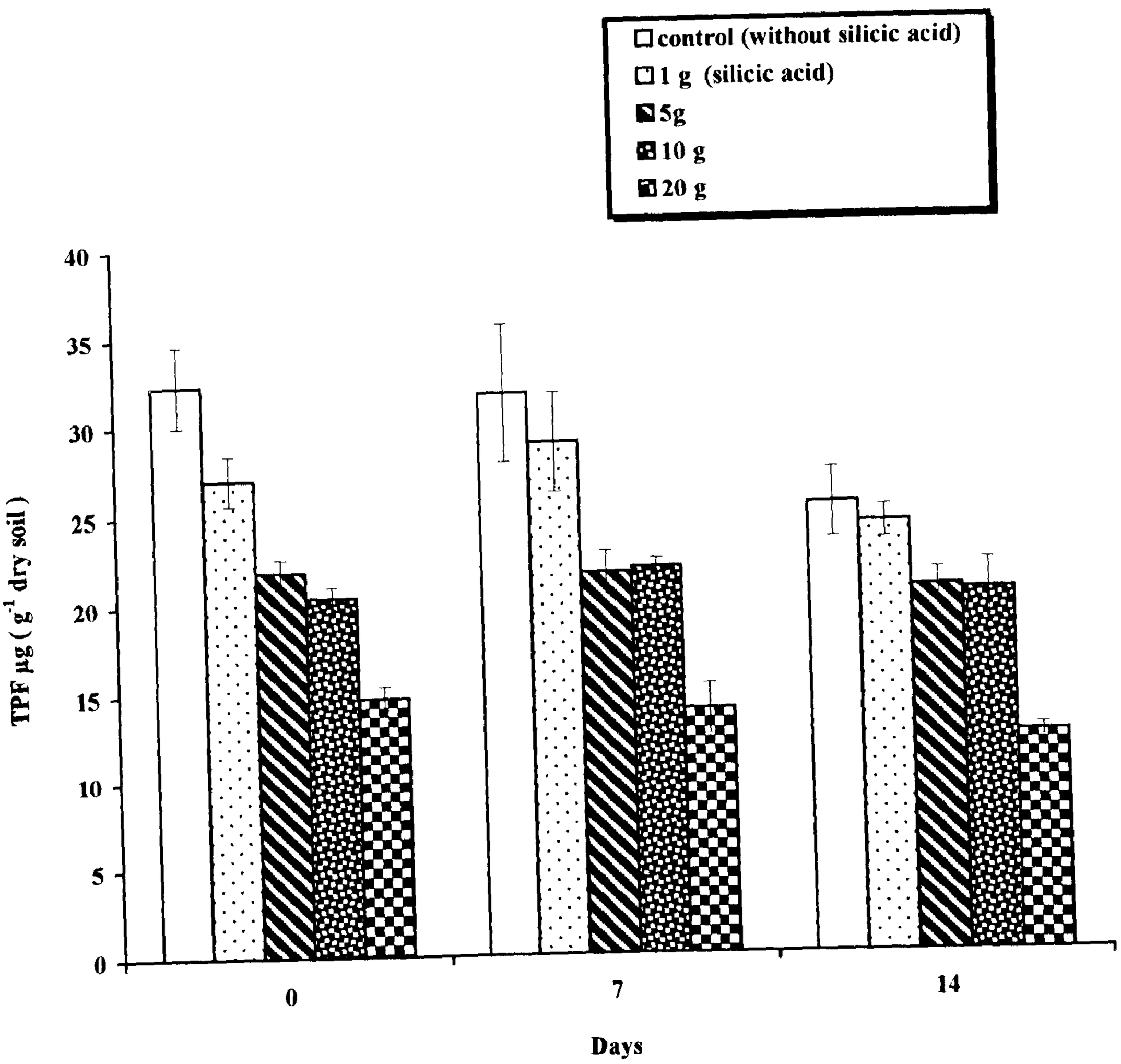

control (without silicic acid)
1.0 g (silicic acid)
5.0 g
10.0 g
20.0 g
µg of p-nitrophenol (g-1 dry soil)
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
0
7
14
21
28
Days

FIG; 5.2

Effect of different amounts of added silicic acid on dehydrogenase activity in agricultural soil. (Means of triplicate, ± standard error).

TPF μg (g^{-1} dry weight $soil^{-1}$).

FIG: 5.2


control (without silicic acid)
1 g (silicic acid)
5g
10 g
20 g
TPF µg (g-1 dry soil)
40
35
30
25
20
15
10
5
0
0
7
14
Days

FIG; 5.3

Effect of different amounts of added silicic acid and sodium silicate on carbon dioxide (CO_2), releases from agricultural soil (mg CO_2 – C 100^{-1} g soil).

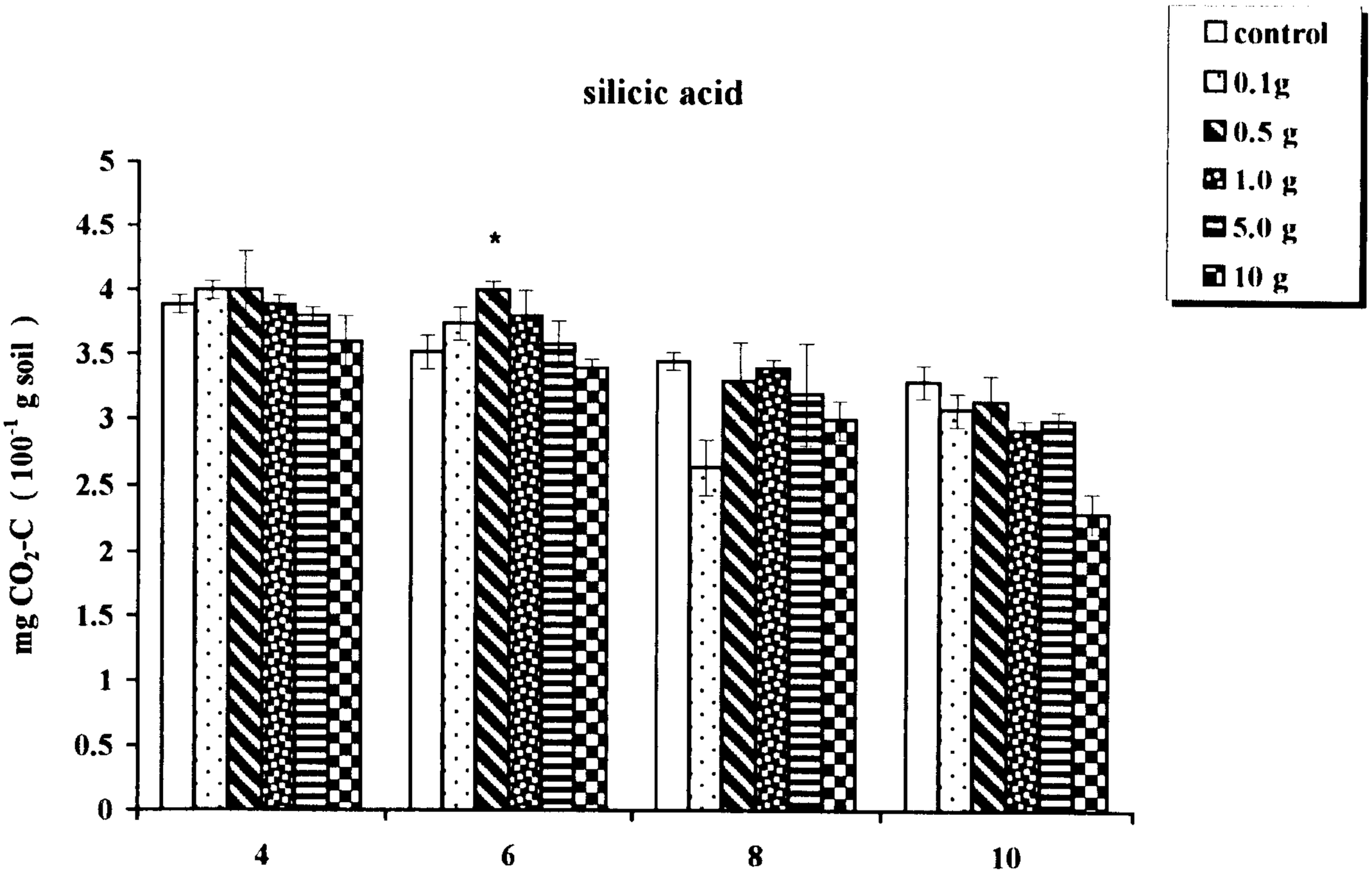
(a) silicic acid amended soil

(b) sodium silicate amended soil.

Means of triplicate, ± standard error. *Significant difference between control and treatment values, $P<0.5$.

FIG: 5.3

(a)


silicic acid
control
0.1g
0.5 g
1.0 g
5.0 g
10 g
mg CO_2-C (100^{-1} g soil)
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
*
*
4
6
8
10

(b)

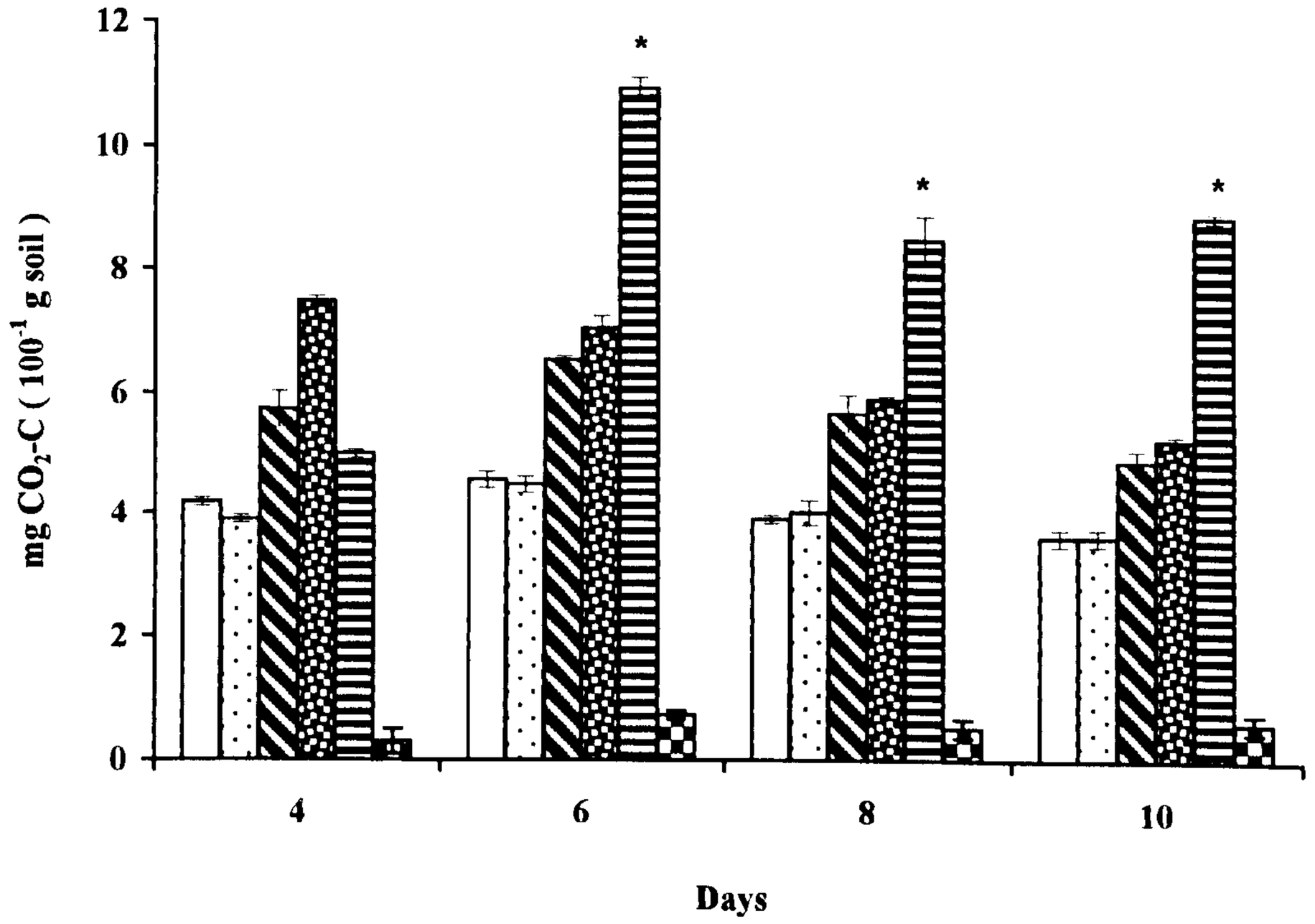

sodium silicate
mg CO_2-C (100^{-1} g soil)
12
10
8
6
4
2
0
*
*
*
4
6
8
10
Days

FIG: 5.4

Determination of soil microbial biomass by chloroform-fumigation-technique in agricultural soil, amended with silicic acid and sodium silicate. (mg carbon 100^{-1} g soil).

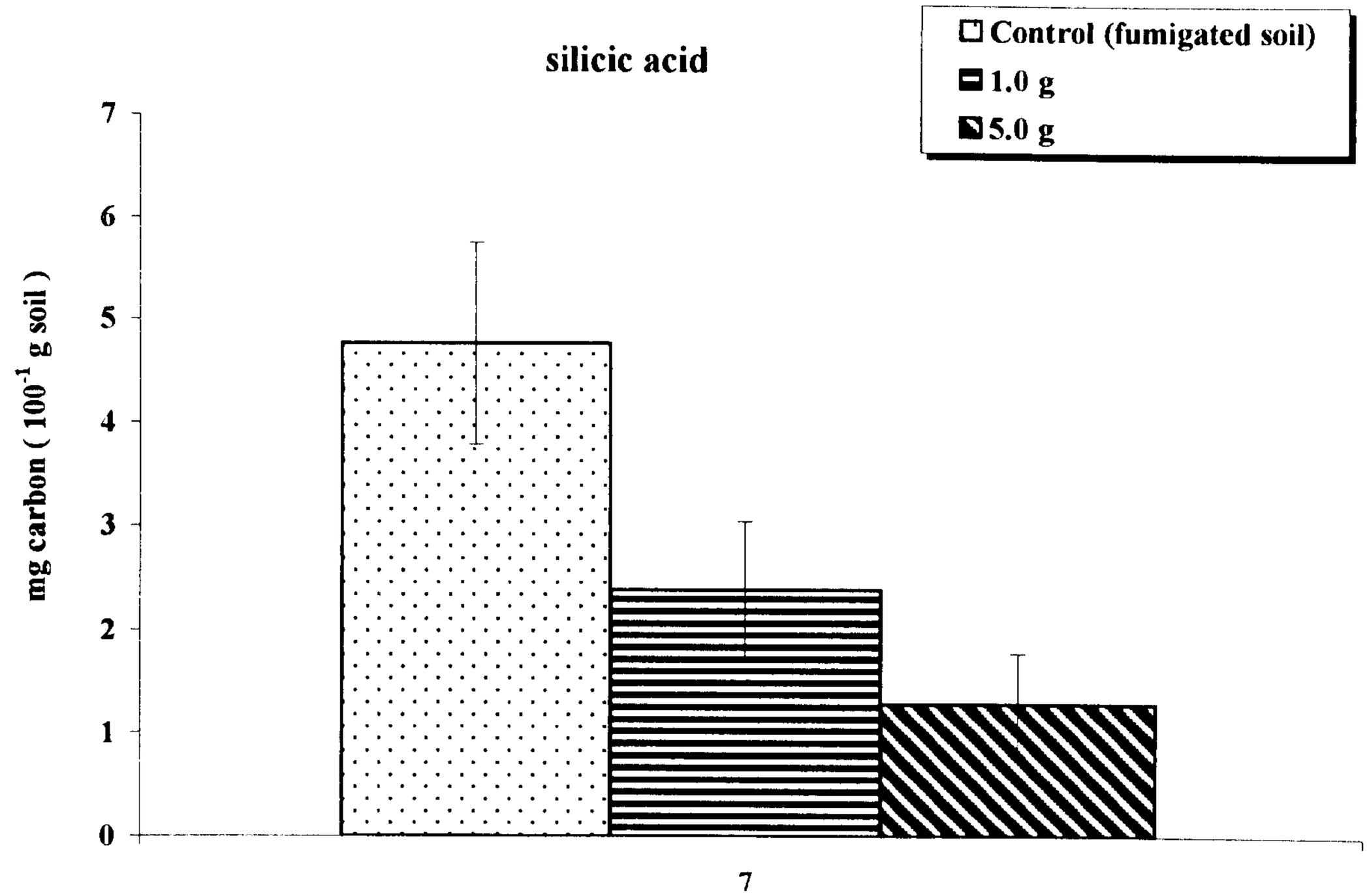
(a) silicic acid amended soil.

(b) sodium silicate amended soil

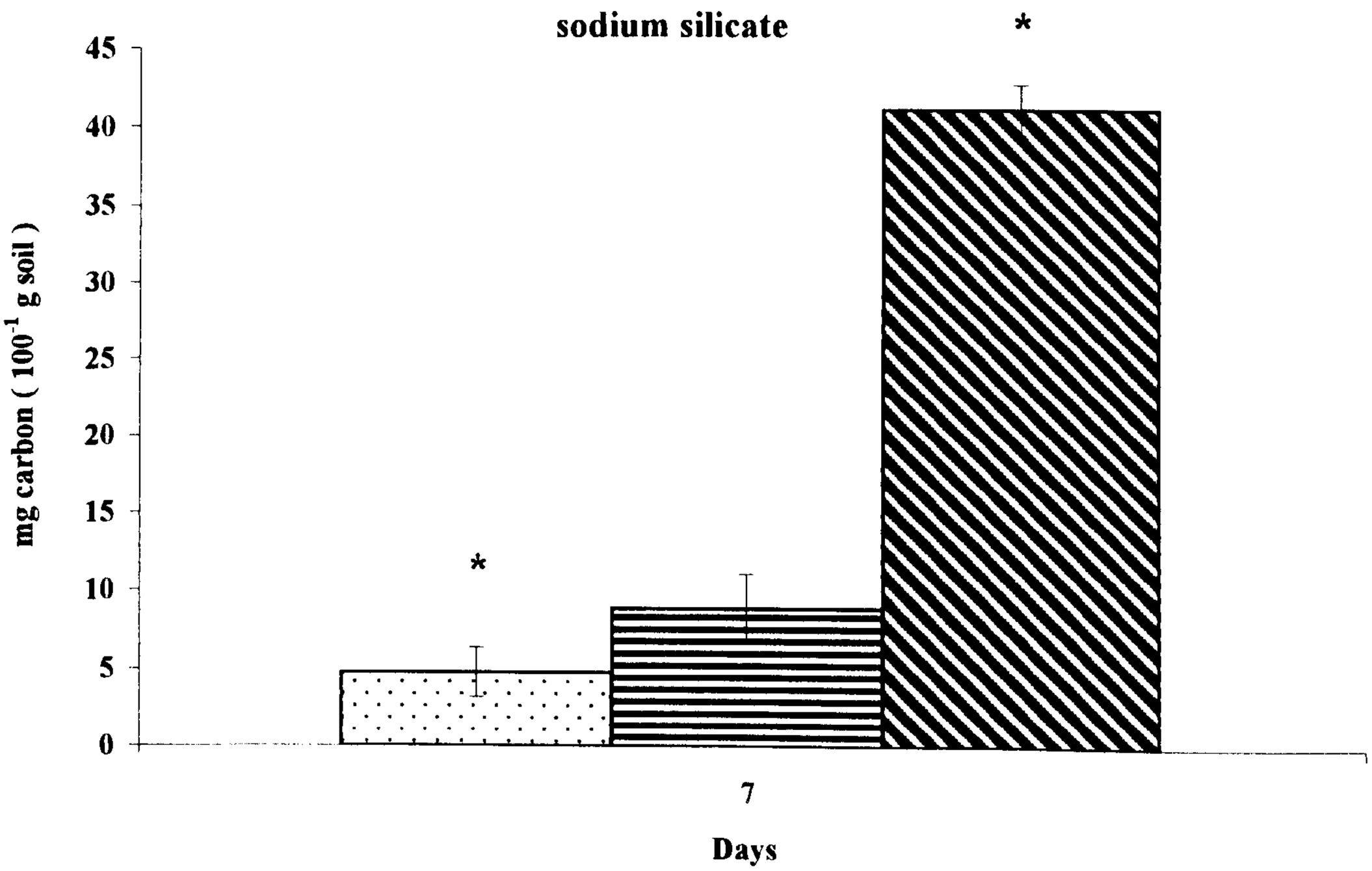
Means of triplicate, ± standard error. *Significant difference between control and treatment values, $P<0.5$.

FIG: 5.4

(a)


silicic acid
Control (fumigated soil)
1.0 g
5.0 g
mg carbon (100-1 g soil)
0
1
2
3
4
5
6
7

(b)


sodium silicate
*
*
mg carbon (100-1 g soil)
0
5
10
15
20
25
30
35
40
45
7
Days

6- GENERAL DISCUSSION

6- GENERAL DISCUSSION

The following overview of the role of silicon in agriculture was synthesized from the Program and Abstracts of the Conference on *Silicon in Agriculture*, Fort Lauderdale, USA, (1999).

Most terrestrial plants grow in media dominated by silicates and the soil solution bathing roots contains Si at concentrations exceeding those of P by approximately a factor of 100. Plant adsorb silicon at rates approximately the same as when they adsorb micro-nutrients. Despite these facts, the plant physiological literature is remarkably devoid of mention of silicon as a plant nutrient. This is largely because silicon is not regarded as an essential nutrient because plants grow in the absence of silicon in nutrient solutions. Silicon-depleted plants however, often differ from those receiving sufficient silicon in the following ways:

(a) chemical composition, (b) mechanical strength (c) yield, (d) disease resistance,

(e) pest resistance, (f) metal toxicity resistance, (g) salt tolerance, and (h) cold hardness.

Although silicon has not been demonstrated to be essential for crop growth it has beneficial effects on the growth of rice, wheat barely, tomato and cucumbers. As a result, Si is applied to crops in several countries to help increase productivity and sustainable production. Plants take up silicon in the form of silicic acid. Silicon becomes particularly important in plants that are stressed e.g., due to: aluminum toxicity, P-deficiency and excess, climatic stress and stresses caused by diseases.

Silicon can control several important diseases of rice, including rice blast, brown spot, sheath blight and leaf scald, but this may be a nutrient effect.

The main agricultural silicon sources are potassium silicate, sodium silicate, calcium silicate. Of particular importance is the use of wollasonite, $CaSiO_3$, which is supplied as a slag by-product from the high temperature production of elemental P. Thermo-phosphate, a Brazilian commercial fertilizer, also supplies large quantities of Si to soils.

Plant-available silicon in soils has been measured in terms of water extractable and phosphate buffer (0.02M, pH 6.95) extractable Si. The critical water-soluble concentration for plant growth is often quoted at 19 $\mu g\ g^{-1}$.

The first experiments using Si as a fertilizer were reported by Liebig in 1840, while Russian soil chemists suggested that SiO_2 and $CaSiO_2$ might be used as fertilizers in 1870.

Since silicon is generally not regarded as an essential nutrient for crop growth it is not surprising that relatively little attention has been given to the chemistry and microbiology of soil silicon. A silicon cycle does not exist, in the environment, instead the only biological involvement in silicon mobilization-immobilization is represented by the solubilization of insoluble silicon, the release of the element from organic-silicon compounds and the immobilization of silicon by bacteria and fungi. In this respect, silicon is similar to phosphorus, P however, is universally regarded as a major plant nutrient.

The literature on the potential role of microbial processes in making silicon available to plants is almost non-existent. The exception being silicate solubilization. This dearth of literature has two implications for work of the nature described in this thesis. Firstly the lack of literature on silicon microbiology means that the field is wide open for study, essentially everything that is done is likely to be novel. On the downside however, the lack of literature means that it is often impossible to extend observations, or explain why phenomena occur. As is evident from the conclusions arrived at here, this means that

the results can often only be speculated upon. The same problem was faced by Al Wajeeh (1999), and commented upon in a thesis, preceding this one from this Laboratory, which detailed an investigation into the *in vitro* microbiology of silicon. This thesis can therefore be seen as an extension of that of Al-Wajeeh-here, studies on the microbiology of silicon is taken from culture into soils.

The essential aims of the work described in this thesis to study a) the microbial solubilization of silicon *in vitro* and in soils, b) the effect of silicon compounds on nitrification, sulphur oxidation, respiration and soil biomass. The silicon compounds used are all potentially useful as sources of fertilizer silicon. A wide range of soils were studied, including the agricultural soils. The variety of soils was included to provide comparisons with the results found when the agricultural loam was studied. It was this soil that was considered in most detail, particularly in relation to silicon and those factors, such as nitrification and S-oxidation, which are important to soil fertility.

Al-Wajeeh (1999) found that silicic acid increased the growth of fungi under oligotrophic and nutrient rich conditions. Under the latter conditions, it also stimulated the growth of a *Streptomyces* species, but decreased the growth of bacteria and yeasts as well as reducing the chlorophyll content of the alga, *Dunaliella parva.* Silicic acid also stimulated the production of silicon by *Aspergillus niger*, but decreased nitrification and sulphur oxidation by this fungus. Silicic acid also reduced antibiotic production by a species of *Streptomyces*.

Based on these results, one might expect an increase in fungal growth in soils, a reduction in bacterial numbers and resultant inhibition of any associated processes, such as nitrification and sulphur oxidation.

The results of the present study show that:

1) Bacteria solubilize rock potash, releasing free silicon into the medium.

2) Growth of a *Penicillium* Sp.*in vitro* increases the solubilization of sodium silicate, but concentrations of free silicon decrease when the fungus is grown in the presence of silicic acid and rock potash, presumably due to Si-immobilization by the fungus.

3) Water-extractable silicon increased when either silicic acid or rock potash was added to all soils, under both aerobic and anaerobic conditions.

4) Liming increased the release of soluble silicon from sodium silicate, silicic acid and rock potash, the effect being seen in all soil types.

5) Silicic acid generally decreased bacterial numbers in all soils, at least over the first 14 days of the incubation period.

6) Silicic acid and rock potash had no effect on nitrification, while the addition of sodium silicate stimulated nitrate production, this effect is assumed to be largely due to the resultant marked increase in soil pH.

7) Addition of silicic acid and rock potash led to increased sulphur oxidation.

8) The addition of silicic acid to the agricultural loam soil led to a decrease in arylsulphatase and dehydrogenase activity, as well as respiration and soil biomass.

Since silicon compounds are added to soils as fertilizers, the obvious question that arises from this study is-To what extent is silicon addition likely to improve, or adversely affect soil fertility?

Firstly, it is clear that water-soluble silicon is released from insoluble silicon compounds following their addition to soil, and that such release is a combination of microbial and chemical-physical processes. The observed decrease in bacterial numbers, arylsulphatase activity, dehydrogenase activity and respiration can be regarded as being detrimental to soil fertility, while increases in sulphur oxidation can be seen as positive responses. The lack of effect of silicon compounds on nitrification can also be seen as being overall desirable; while increased nitrate following the addition of sodium silicate can be regarded a damaging because it leads to the, above-mentioned loss, of N from soils. It is probable that many of the observed effects on soils fertility of the silicon compounds used here relate more to their marked alkaline nature, than to any direct effect of silicon itself.

In conclusion, the view that silicon compounds, and the silicon ion itself, is inert and unlikely to influence soil processes is not verified by the results presented here. As a result, it is recommended that as much consideration should be given to the potential negative effects on soil of silicon fertilization as is given, for example, to when pesticides are applied to soils.

SUGGESTIONS FOR FUTURE WORK

Since the principle aim of the research described here was to determine what effect silicon compounds have on soil fertility the obvious next step would be to extend this work to processes such as asymbiotic and symbiotic N-fixation, denitrification, the breakdown of leaf liter or crop residues and finally aspects of the mineralization and solubilization of phosphorus. Such effects could be determined in the laboratory and /or the field.

It would be desirable to determine what factors influence the microbial solubilization of silicon compounds (and rocks) to determine if (a) such solubilization occurs under low nutrient conditions likely to be met with in natural environments and (b) to determine if organic acids or chelators are actually involved in this process, particularly under such oligotrophic conditions.

The fact that silicon is not a major plant nutrient means that the involvement of microorganisms in influencing its availability to plants, and the effects of fertilizer silicon on soil properties is likely to continued to be neglected by funding agencies as being of no great importance. However, the fact that US companies such as Albright Wilson, the PQ Corporation and the Calcium Silicate Corporation fund meetings organized by the "US Silicon in Agriculture Organizing Committee" suggests that commercial and academic interest in silicon may increase in the future.

7. APPENDIX

7- APPENDIX (A)

7.1 Solid and liquid media and chemicals used, for culturing and soil analysis.

A) MEDIA

(a) Czapek Dox Agar (modified)	**(Oxoid)**
Typical formula (g/L)	
1. Sodium nitrate	2.0
2. Potassium chloride	0.5
3. Magnesium glycerophosphate	0.5
4. Ferrous sulphate	0.01
5. Potassium sulphate	0.35
6. Sucrose	30.0
7. Agar	12.0

pH to 6.8 ± 0.2

A proprietary formulation (Oxoid) of Czapek Dox Agar was used specially for fungal growth. It prepared by suspending 45.4 g of the powder in a litre of distilled water. The medium was dissolved and sterilized by autoclaving at 121^{0}C for 15 min.

(b) Czapek Dox liquid medium (modified)	**(Oxoid)**
Typical formula (g/L)	
1. Sodium nitrate	2.0

2. Potassium chloride	0.5
3. Magnesium glycerophosphate	0.5
4. Ferrous sulphate	0.01
5. Potassium sulphate	0.35
6. Sucrose	30.0

pH 6.8 ± 0.2

This liquid medium was prepared by suspending 33.4 g of the powder in one litre of distilled water. The medium was then dissolved and sterilized by autoclaving at 121^0C for 15 min.

(c) <u>Nutrient Agar</u> **(Oxoid)**

1. 'Lab-lemco' powder	1.0
2. Yeast extract	2.0
3. Peptone	5.0
4. Sodium chloride	5.0
5. Agar	15.0

pH 7.4 ± 0.2

Nutrient Agar is a basic medium used to subculture organisms for maintenance purposes or to check the purity of subcultures from isolation plates prior to biochemical or serological tests. This medium can be used for the cultivation of organisms. The

medium was prepared by suspending 28g in litre of distilled water, boiled to dissolve completely and sterilized by autoclaving at 121^0C for 15 minutes.

(d) <u>Nutrient broth</u> **(Oxoid)**

1. 'Lab-lemco' powder	1.0
2. Yeast extract	2.0
3. Peptone	5.0
4. Sodium chloride	5.0

pH 7.4 ± 0.2

A general purpose liquid medium for the cultivation of micro-organisms not exacting in their nutritional requirements. Medium was prepared adding 13g to 1 litre of distilled water, mixed well and distributed into final containers It was then sterilized by autoclaving at 121^0C for 15 minutes.

<u>(e) Plate count Agar</u>

(Oxoid)

1. Tryptone	5.0
2. Yeast extract	2.5
3. Glucose	1.0
4. Agar	9.0

pH 7.0 ± 0.2

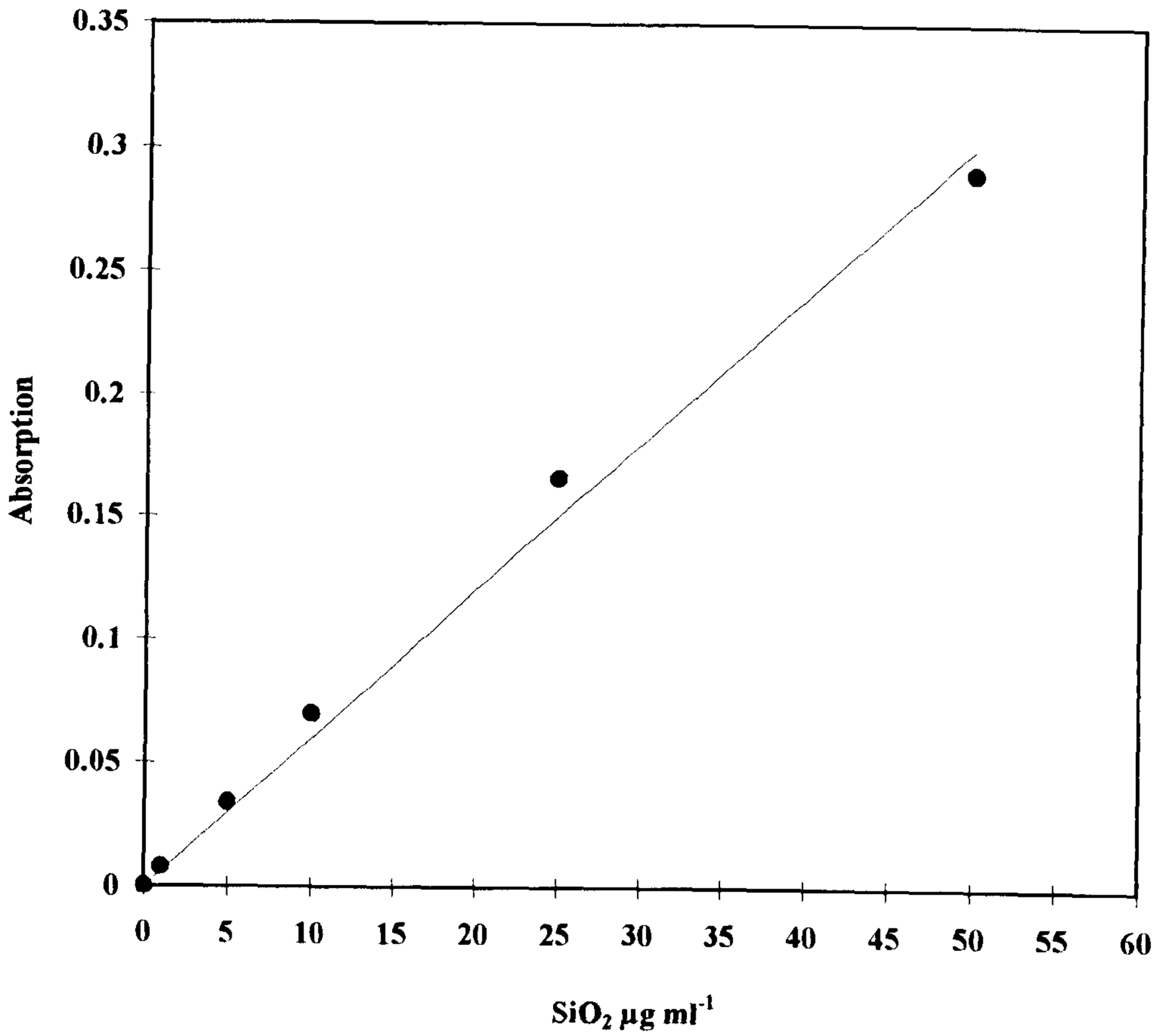
A medium used for the enumeration of viable organisms particularly in milk and dairy products. This medium was prepared by adding 17.5g to 1 litre of distilled water and dissolved by boiling with frequent stirring, and distributed into final containers. Medium was then sterilized by autoclaving at 121^0C for 15 minutes.

B) <u>CHEMICALS</u>

(1) Ammonium molybdate	(BDH)
(2) Ammonium sulphate $(NH_4)_2\ SO_4$	(BDH)
(3) Ascorbic acid	(BDH)
(4) Acetone	(BDH)
(5) Acetone (analytical reagent)	(Fisher)
(6) Acetic acid glacial	(BDH)
(7) Barium chloride, 2 hydrate ($BaCl_2.\ 2H_2O$)	(BDH)
(8) Calcium chloride	(BDH)
(9) Chromotropic acid ($C_{10}\ H_6\ O_8\ S_2\ Na_2$)	(BDH)
(10) Chloroform ($CHCl_3$)	(BDH)
(11) Elemental sulphur	(Fisons)
(12) Ethanol (absolute)	(-------)
(13) Gum acacia	(BDH)
(14) Glucose	(BDH)
(15) Hydrochloric acid (conc:)	(BDH)
(16) Lithium chloride	(BDH)
(17) N-(1-naphthyl)-ethylene diamine dihydrochloride	(BDH)
(18) Oxalic acid	(sigma)
(19) Phenolphthalein (Indicator)	(sigma)

(20) Phenol	(BDH)
(21) *p*-nitrophenyl sulphate	(sigma)
(22) Potassium chloride (KCl)	(BDH)
(23) Potassium hydroxide	(BDH)
(24) Potassium nitrate (KNO_3)	(Fisons)
(25) Potassium silicate	(Pro labo)
(26) Ringer's solution ¼ strength	(BDH)
(27) Rock potash	(--------)
(28) Silicic acid	(sigma)
(29) Sodium acetate trihydrate	(sigma)
(30) Sodium fluorosilicate (EIL SILICA Standard 1000 ppm)	(BDH)
(31) Sodium hydroxide	(BDH)
(32) Sodium hypochloride	(Fisons)
(33) Sodium silicate	(Riedel-de Haën)
(34) Sodium nitrite ($NaNO_2$)	(sigma)
(35) Standard *p*-nitrophenol	(sigma)
(36) Sodium sulphate anhydrous ($NaSO_4$)	(BDH)
(37) Sulphuric acid (H_2SO_4 conc:)	(Fisher)
(38) Sulphanilamide	(sigma)
(39) Tris (hydroxy methyl)-aminomethane	(BDH)
(40) Triphenyltetrazolium chloride (TTC)	(sigma)
(41) Triphenylformazan (TPF)	(TPF)
(42) Toluene	(BDH)

STANDARD CURVE FOR SILICON



APPENDIX (B)

7.2 ANALYTICAL METHODS

(A) Method used for the detection of free silicon

Soluble silicon in the solution was analysed by spectrophotometer, adding, to 1 ml of filtrate: ammonium molybdate (2 ml, 10% w/v), ascorbic acid (2 ml, 5% w/v), 1 ml oxalic acid (1 ml, 10% w/v), and concentrated hydrochloric acid (5 ml, 1:1 diluted with distilled water), and mixed thoroughly. The intensity of the blue colour was measured at 600 nm using a spectrophotometer.

Analysis of inorganic N-ions.

(B) Indophenol blue method for the determination of ammonium-N (NH_4^+-N) (Wainwright & Pugh 1973).

To filtrate (2 ml) was added, distilled water (7 ml), *phenolate reagent (5 ml), and sodium hypochlorite (5 ml) solution (0.9% v/v active chlorine), mixed and incubated at 25^0C for 20 minutes in the dark. The intensity of indophenol-blue-ammonium-complex was measured at 630 nm using a spectrophotometer. The concentration of NH_4^+-N was determined by reference to a standard curve (0-50 µg NH_4^+-N ml^{-1}) prepared from a standard solution of ammonium sulphate $(NH_4)_2 SO_4$.

(a) <u>Standard ammonium solution</u>: was prepared by dissolving 0.4717 g ammonium sulphate $(NH_4)_2SO_4$ in 1 litre distilled water for (100 µg NH_4^+-N ml^{-1}).

(b) <u>Phenol solution:</u> was prepared by dissolving phenol (62.5 g) in ethanol (25 ml) and adding acetone (18.5 ml) to give a total of 100 ml. The phenol solution was stored in the dark at 4^0C.

(c) *<u>Phenolate reagent:</u> was prepared by mixing 20 ml of phenol solution with 20 ml caustic solution (27% NaOH w/v) and diluting to 100 ml. The reagent was prepared fresh daily.

(C) Analysis of nitrite-N (NO_2^--N). (Hesse, 1971)

Filtrate (2 ml) was added to a 50 ml volumetric flask, diluted with distilled water (40 ml) and *<u>diazotising reagent</u> (1 ml) was added and incubated at room temperature for 5 minutes. **<u>Coupling reagent</u> (1 ml) was added and the volume was made up to mark with distilled water. After 20 minutes incubation at room temperature, the intensity of the pink colour formed was measured at 520 nm using a spectrophotometer and the amount of nitrite was determined by reference to a calibration curve (0-10 µg NO_2^--N ml^{-1}) prepared from a standard solution of $NaNO_2$.

(a) <u>Standard nitrite solution:</u> was prepared by dissolving 0.4929 g sodium nitrite $NaNO_2$ (100 µg NO_2^--N ml^{-1}) in 1 litre distilled water volumetrically.

(b) * <u>Diazotising reagent:</u> 0.5g of sulphanilamide was added to 2.5 N HCl (100 ml) and dissolved. The reagent was stored in an amber bottle in refrigerator at 4^0C.

(c) **<u>Coupling reagent:</u> 0.3 g of N-(1-naphthyl)-ethylenediamine hydrochloride was dissolved in 0.1 N HCl (100 ml). The reagent was stored in an amber bottle in refrigerator at 4^0C.

(D) Chromotropic acid method for nitrate-N determination (Sims and Jackson, 1971).

To filtrate (3 ml), *chromotropic acid reagent (7 ml) were added, mixed, cooled in cold water and incubated at 40^0C for 45 minutes. The intensity of the yellow CTA-NO_3 complex was measured at 430 nm using a spectrophotometer. The NO_3^- - N concentration was determined by reference to a standard curve (0-5 µg NO_3^- -N ml^{-1}), prepared from a standard solution of KNO_3.

(a) <u>Standard Nitrate Solution:</u> 0.722 g Potassium nitrate (KNO_3) were dissolved in distilled water and made up to 1 litre volumetrically, for 100 µg NO_3^- -N ml^{-1}.

(b) <u>Chromotropic acid reagent</u> (C_{10} $H_6O_8S_2$ Na_2):

A 0.1% (v/v) stock solution of chromotropic acid in concentrated sulphuric acid (H_2SO_4) was prepared by dissolving 1.84g chromotropic acid in litre H_2SO_4 (1:1). This solution was stored in an amber bottle in refrigerator at 4^0C for several months.

(c) *A working chromotropic acid solution (CTA):

A working CTA-solution (0.01% v/v) was prepared by diluting 100 ml of stock solution to 990 ml with concentrated sulphuric acid (H_2SO_4) then added 10 ml concentrated HCl using fume cupboard. This solution was stored at 4^0C for several weeks only.

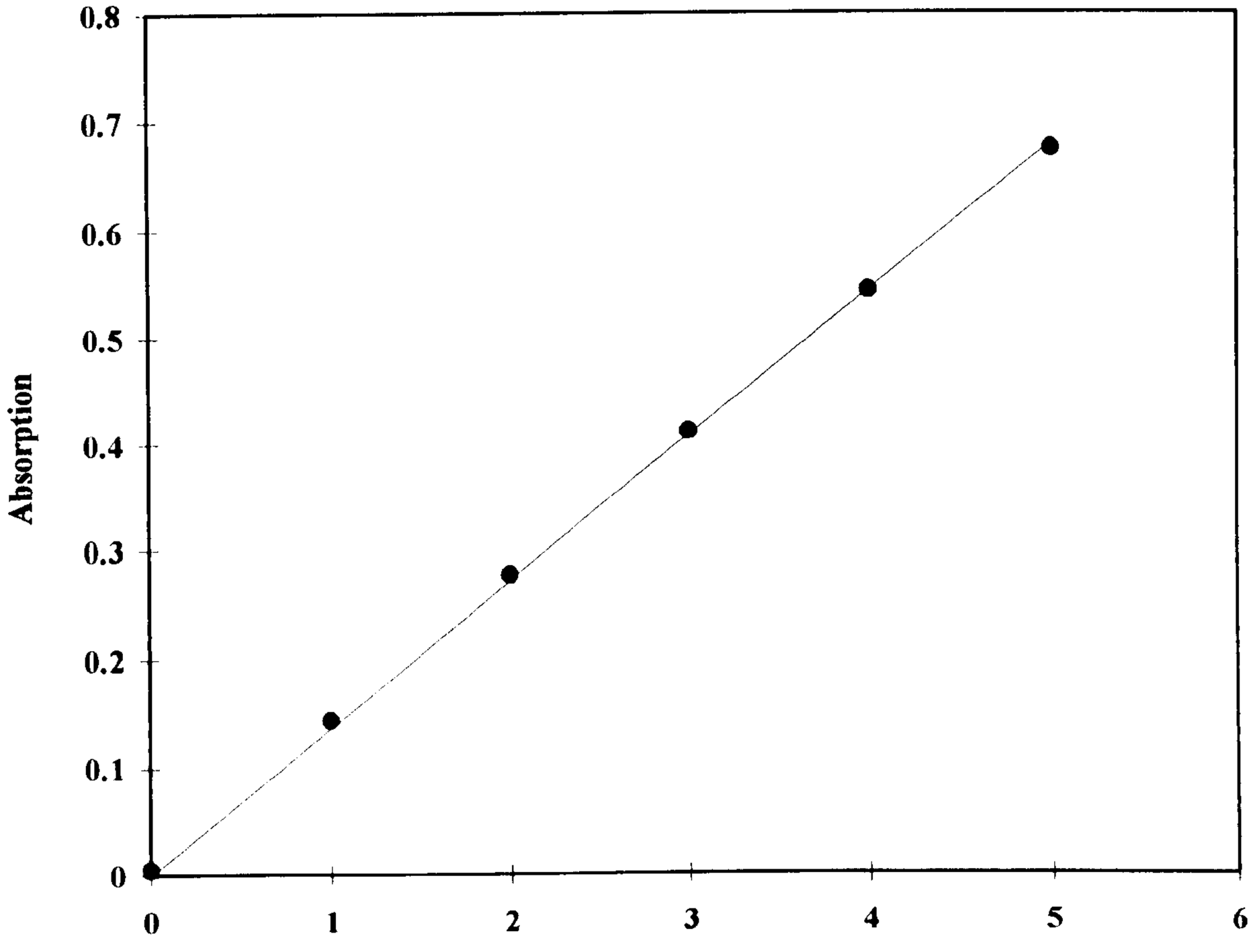
Analysis of inorganic S-ions

(E) Turbidimetric analysis of sulphate-S (Hesse, 1971).

To 5ml filtrate in 25ml volumetric flask, 1g $BaCl_2.2H_2O$ and 2ml of gum-acacia (0.25%w/v), were added and mixed, and the volume was up-to mark with distilled water. The white suspension resulting from precipitation of barium sulphate, was measured at 470 nm by spectrophotometer. The concentration of SO_4^{2-} - S was determined by reference to a standard curve (0 –100 µg SO_4^{2-} - S ml^{-1}) prepared from a standard solution of $Na_2SO_4.10H_2O$.

(b) Standard sulphate-S solution: 0.443 grams of sodium sulphate ($Na_2SO_4.10H_2O$), were dissolved in distilled water 1 litre, which gives the concentration 100 µg SO_4^{2-} - S ml^{-1}.

STANDARD CURVE FOR ARYLSULPHATASE



(F) Arylsulphatase assay.

To soil (1g), containing in universal vials (25 ml), 4 ml acetate buffer, 0.25 ml toluene and 1 ml *p*-nitrophenyl sulphate solution were pipette, swirled for few seconds to mix the contents, screwed on top and placed in a water bath at 25^0C. After 1 hr vials were removed from the water bath, and 1ml $CaCl_2$ (0.5 M), 4 ml NaOH (0.5 M) were added, vials were swirled for few seconds, and the soil suspension was filtered through Whatman No.1 folded filter paper into a test tube.

Controls were performed with each soil sample analyzed to allow for colour not derived from *p*-nitrophenol released by arylsulphatase activity. For the control, above procedure was followed except 1 ml of *p*-nitrophenyl sulphate solution. After incubation at 25^0 C for 1 hr, 1 ml of $CaCl_2$ (0.5 M) and 4 ml of NaOH (0.5 M) were added and then 1 ml of *p*-nitrophenyl sulphate solution was added immediately before filtration of the soil suspension into a test tube. Filtrate was transferred to a 4 ml cuvette and intensity of the yellow colour was measured at 400 nm in the spectrophotometer against a reagent blank.

Preparation of chemicals

(a) Tris-HCl buffer (100 mM)

12.1 gram of Tris (hydroxy methyl) aminomethane were dissolved in 700 ml distilled water, pH was adjusted at 7.6 (pH of the soil was 7.3, between the range of 6 to 7.5) and added more distilled water up to 1000 ml.

(b) Triphenyltetrazolium chloride (TTC) solution

1g of triphenyltetrazolium chloride (TTC) was dissolved in 80 ml tris-buffer and volume was made up with the same buffer to 100 ml.

(c) Extractant

Acetone (analytical grade)

TPF standard solution

50 mg of Triphenyl formazan (TPF) were dissolved in 80 ml of acetone (500µg TPF ml^{-1}) and the volume was made up to 100 ml with acetone. Field-moist agriculture soil (5 g) was weighed into 60 ml glass bottles and mixed with 5 ml triphenyltetrazolium chloride (TTC) solution. All the bottles were sealed with solvent resistant-rubber stoppers and incubated for 24 hours at 30^0 C. The control contains only 5 ml tris-buffer (without TTC). After the incubation, 40 ml acetone was added to each bottle and the bottles were shaken thoroughly and further incubated at room temperature for 2 hours in the dark (shaking the tubes at intervals). The soil suspension (15 ml) was then filtered through Whatman No.1 folded filter paper and the optical density of the clear supernatant was measured against the blank (8.3 ml tris buffer pH 7.3 in 50 ml volumetric flask and the volume was made with acetone up to 50 ml mark).

Titration-method

After incubation, KOH (10 ml, 0.1M) solution were transferred to 100 ml Erlenmeyer flask. Into the Erlenmeyer flask, $BaCl_2 . 12H_2O$ (2 ml, 0.5 M) were added. This is used to precipitate the potassium carbonate to prevent over-estimation of the titration end-point.

Phenolphthalein indicator (3 drops) were added and the solution was titrated with 0.1M HCl to end-point by using 25 ml burette. Blanks were also titrated of 10 ml fresh KOH.

Preparation of Barium chloride solution (0.5 M)

122.14 g of $BaCl_2 \cdot 2H_2O$ were dissolved in distilled water and volume was made up with distilled water to 1 litre.

Preparation of phenolphthalein (0.1%) indicator.

Phenolphthalein powder (0.1 g) was dissolved in ethanol (80 ml 60% v/v) and the volume of ethanol was increased up to 100 ml.

Chloroform (ethanol-free $CHCl_3$)

Commercial chloroform was washed with about 5% by volume H_2SO_4 (conc:) by shaking in a separating funnel to separate off the acid, and re-washed with 10 rinses of distilled water. (Ethanol-free $CHCl_3$ was stored in the dark to prevent photochemical build-up of explosive-by products). Ethanol-free $CHCl_3$ is recommended because ethanol cannot be completely removed from soil after fumigation (Jenikinson 1988) and it is used as a substrate, mineralized to CO_2, and thus is incorrectly measured as biomass C.

The CO_2 evolved during the incubation was measured by the Titration method, as described above.

TABLE (7.1) **SOIL CHARACTERISTICS**

Content	D	A	F	C
Total organic matter (% w/w)	16.2	8.9	16.0	20.4
Total organic carbon (% w/w)	12.0	3.8	13.0	20.0
Total organic nitrogen (% w/w)	1.06	0.4	0.6	2.0
pH	5.2	6.5	6.2	4.9

Abbreviations: D. Deciduous, A. Agricultural, F. Fern, and C. Coniferous.

All soil samples were collected by digging about 10 cm deep from soil surface.

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8-REFERENCES

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